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ORIGINAL ARTICLE

Fibroblast growth factor 23 independently predicts adverse outcomes after an acute coronary syndrome

Andrea Kallmeyer[1,2], Ana Pello[1,2], Ester Cánovas[1], Álvaro Aceña[1,2], María Luisa González-Casaus[3], Nieves Tarín[4,5], Carmen Cristóbal[5,6], Carlos Gutiérrez-Landaluce[6], Ana Huelmos[7], Aida Rodríguez-Valer[2], Óscar González-Lorenzo[1,2], Joaquín Alonso[8], Lorenzo López-Bescós[5], Jesús Egido[9,10,11], Ignacio Mahillo[12], Óscar Lorenzo[2,9,11] and José Tuñón[1,2,11,13]*

[1]*Department of Cardiology, IIS-Fundación Jiménez Díaz, Madrid, Spain;* [2]*Faculty of Medicine, Autónoma University, Madrid, Spain;* [3]*Department of Laboratory Medicine, Unit of Mineral Metabolism, Hospital La Paz, Madrid, Spain;* [4]*Department of Cardiology, Hospital Universitario de Móstoles, Madrid, Spain;* [5]*Faculty of Medicine, Rey Juan Carlos University, Madrid, Spain;* [6]*Department of Cardiology, Hospital Universitario de Fuenlabrada, Madrid, Spain;* [7]*Department of Cardiology, Hospital Universitario Fundación Alcorcón, Madrid, Spain;* [8]*Department of Cardiology, Hospital de Getafe, Madrid, Spain;* [9]*CIBERDEM, Madrid, Spain;* [10]*Department of Nephrology, IIS-Fundación Jiménez Díaz, Madrid, Spain;* [11]*Laboratory of Vascular Pathology, IIS-Fundación Jiménez Díaz, Madrid, Spain;* [12]*Laboratory of Epidemiology, IIS-Fundación Jiménez Díaz, Madrid, Spain; and* [13]*CIBERCV, ISCIII, Madrid, Spain*

Abstract

Aims Abnormalities of mineral metabolism (MM) have been related to cardiovascular disorders. There are no reports on the prognostic role of MM after an acute coronary syndrome (ACS). We aim to assess the prognostic role of MM after an ACS.

Methods and results Plasma levels of components of MM [fibroblast growth factor 23 (FGF23), calcidiol, parathormone, klotho, and phosphate], high-sensitivity C-reactive protein, and N-terminal-pro-brain natriuretic peptide were measured in 1190 patients at discharge from an ACS. The primary outcome was a combination of acute ischaemic events, heart failure (HF) and death. Secondary outcomes were the separate components of the primary outcome. Age was 61.7 ± 12.2 years, and 77.1% were men. Median follow-up was 5.44 (3.03–7.46) years. Two hundred and ninety-four patients developed the primary outcome. At multivariable analysis FGF23 (hazard ratio, HR 1.18 [1.08–1.29], $P < 0.001$), calcidiol (HR 0.86 [0.74–1.00], $P = 0.046$), previous coronary or cerebrovascular disease, and hypertension were independent predictors of the primary outcome. The predictive power of FGF23 was homogeneous across different subgroups of population. FGF23 (HR 1.45 [1.28–1.65], $P < 0.001$) and parathormone (HR 1.06 1.01–1.12]; $P = 0.032$) resulted as independent predictors of HF. FGF23 (HR 1.21 [1.07–1.37], $P = 0.002$) and calcidiol (HR 0.72 [0.54–0.97), $P = 0.028$) were independent predictors of death. No biomarker predicted acute ischaemic events. FGF23 predicted independently the primary outcome in patients with estimated glomerular filtration rate > 60 mL/min/1.73 m^2.

Conclusions FGF23 and other components of MM are independent predictors of HF and death after an ACS. This effect is homogeneous across different subgroups of population, and it is not limited to patients with chronic kidney disease.

Keywords Acute coronary syndrome; Calcidiol; Cardiovascular risk; Chronic kidney disease; Fibroblast growth factor 23; Mineral metabolism; Parathormone

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**Correspondence to: José Tuñón, Department of Cardiology, Fundación Jiménez Díaz, Avda. Reyes Católicos 2, 28040 Madrid, Spain. Email: jtunon@fjd.es*

Introduction

Mineral metabolism (MM) is primarily known by its role in maintaining mineral homeostasis. When renal function declines below an estimated glomerular filtration rate (eGFR) of 75 mL/min/1.73 m^2, the ability of the kidneys to eliminate phosphate decreases.[1] Then, an increase in bone production of fibroblast growth factor 23 (FGF23) happens, which promotes phosphate elimination. Additionally, FGF23 leads to a decrease in vitamin D (calcidiol) levels due to both an enhanced catabolism and a reduction in its production.[1,2] Furthermore, the decrease in calcidiol levels releases parathormone (PTH) by feedback inhibition, leading to an increase in its levels. Although this compensatory mechanism,

which has been called the canonical pathway of FGF23, protects the organism against the adverse cardiovascular effects of phosphate accumulation, these abnormalities are also associated with cardiovascular damage. In this regard, reduced levels of calcidiol and high PTH plasma levels are related with hypertension, left ventricular hypertrophy, coronary artery disease (CAD), stroke, and with an elevated cardiovascular risk.[3–11] Similarly, high FGF23 plasma levels also promote left ventricular hypertrophy and fibrosis, endothelial dysfunction and vascular calcification, and they increase mortality and cardiovascular events.[12–16] Of interest, harmful FGF23 actions appear mainly when over-physiological concentrations of this molecule are present, so it binds to receptors different of the canonical FGFR1 and its co-receptor klotho.[17] The soluble form of klotho has been said to have cardio-protective and anti-aging properties.[18]

Despite its relationship with renal function, there is growing evidence that shows that the canonical pathway of FGF23 is not the unique one leading to cardiovascular damage. Concerning to it, we have seen that abnormalities of MM may be present in patients with normal eGFR.[19] Moreover, we and others have demonstrated that increased levels of FGF23 may predict the development of cardiovascular events in patients with stable CAD with average renal function.[16,20] It has been demonstrated that FGF23 can be produced in a paracrine manner by cardiac myocardial cells, enhancing the activity of cardiac fibroblasts and promoting fibrosis independently of Klotho and FGFR1. This non-canonical pathway of FGF23 may explain its involvement in cardiac conditions even though chronic kidney disease (CKD) is not present. Then, it seems that the cardiovascular implications of MM abnormalities are not restricted to patients with CKD.

Despite the potential relevance of MM in cardiovascular disease, there are no reports on its possible prognostic role in patients with an acute coronary syndrome (ACS). Thus, in this work, we studied 1190 patients with ACS to ascertain if plasma levels of the MM components (calcidiol, FGF23, PTH, phosphate, and klotho) have a predictive capacity in this clinical setting.

Methods

Patients

We analysed the population of the BACS & BAMI (Biomarkers in ACS & Biomarkers in Acute Myocardial Infarction) study. It included patients admitted to five hospitals in Madrid with either non-ST elevation acute coronary syndrome (NSTEACS) or ST-elevation myocardial infarction (STEMI). Inclusion and exclusion criteria have been defined previously.[21]

Between July 2006 and June 2014, 2740 patients were discharged from the study hospitals with a diagnosis of NSTEACS or STEMI; 1483 patients were excluded due to the following prespecified criteria: age over 85 years (16.4%), presence of disorders or toxic habits limiting survival (29.8%), impossibility to perform cardiac revascularization (9.6%), coexistence of other significant cardiopathy (5.7%), impossibility to perform follow-up (16.3%), clinical instability beyond the sixth day after the index event (10.9%), refusal to participate in the study (1.5%), and impossibility of the investigators to include them (9.8%). From the included 1257 patients, 1230 completed the follow-up. From these, 1190 had an adequate assessment of the components of mineral metabolism. On admission, clinical variables were recorded, and plasma was taken for analysis. Last follow-up visits were carried out in June 2016.

Ethics statement

The research protocol suited the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the human research committees of the institutions participating in this study: Fundación Jiménez Díaz University Hospital, Fundación Alcorcón Hospital, Fuenlabrada Hospital, Puerta de Hierro Majadahonda University Hospital, and Móstoles University Hospital. All patients signed informed consent document. The date of approval by the Ethics Committee was 24 April 2007 (act number 05-07).

Study design

At baseline, clinical variables were recorded, and 12-h fasting venous blood samples were withdrawn and collected in EDTA. Blood samples were centrifuged at 2500 *g* for 10 min, and plasma was stored at −80°C. Patients were seen every year at their hospitals. At the end of follow-up, the medical records were reviewed, and patient status was confirmed by telephone contact.

The primary outcome was the combination of acute ischaemic events (STEMI, non-STEMI, unstable angina, transient ischaemic attack, and stroke), HF (both new onset HF and decompensated HF), and all-cause mortality. Secondary outcomes were the components of the primary outcome: acute ischaemic events, HF, and death. NSTEACS was defined as rest angina lasting more than 20 min in the previous 24 h, or new-onset class III-IV angina, along with transient ST depression or T wave inversion in the electrocardiogram considered diagnostic by the attending cardiologist and/or troponin elevation. STEMI was defined as symptoms compatible with angina lasting more than 20 min and ST elevation in at least two adjacent leads in the electrocardiogram without response to nitro-glycerine and troponin ele-

vation. A previous acute myocardial infarction was diagnosed in the presence of new pathological Q waves in the electrocardiogram along with a concordant new myocardial scar identified either by echocardiography or nuclear magnetic resonance imaging.[22] Stroke was defined as rapid onset of a neurologic defect attributable to a focal vascular territory lasting more than 24 h or confirmed by new cerebral ischaemic lesions on imaging studies. Transient ischaemic attack was defined as transient neurological signs and symptoms of cerebral ischaemia but resolved within the first 24 h and without cerebral acute ischaemic lesions at imaging techniques.

Although all events were recorded for each case, patients were excluded from the Cox regression analysis after the first event. Then, despite the total number of events is also described, patients that had more than one event computed only once for these analyses. Patient's follow-up was rigorous, made by clinical visits and also by telephone contact and hospital records reviews.

Biomarker and analytical studies

Plasma determinations were performed at the laboratory of Mineral Metabolism at La Paz Hospital and at the laboratories of Vascular Pathology and Biochemistry at Fundación Jiménez Díaz University Hospital. The investigators who performed the laboratory studies were unaware of clinical data. Plasma calcidiol levels were quantified by chemiluminescent immunoassay on the LIAISON XL analyser (LIAISON 25OH-Vitamin D total Assay DiaSorin, Saluggia, Italy). FGF23 was measured by an enzyme-linked immunosorbent assay that recognizes epitopes within the carboxyl-terminal portion of FGF23 (Human FGF23, C-Term, Immutopics Inc, San Clemente, CA), and klotho levels were measured by ELISA (Human soluble alpha klotho assay kit, Immuno-Biological Laboratories Co., Hokkaido, Japan). Intact PTH was analysed by a second-generation automated chemiluminescent method (Elecsys 2010 platform, Roche Diagnostics, Mannheim, Germany), and phosphate was determined by an enzymatic method (Integra 400 analyser, Roche Diagnostics, Mannheim, Germany). N-Terminal pro-brain natriuretic peptide (NT-proBNP) levels were assessed by immunoassay (VITROS, Orthoclinical Diagnostics, Raritan, NJ, USA), high-sensitivity C-reactive protein (hs-CRP) by latex-enhanced immunoturbidimetry (ADVIA 2400 Chemistry System, Siemens, Munich, Germany), and troponin by immunometric immunoassay with a mice biotin-monoclonal antibody and a luminescent reaction (Ortho Clinical Diagnostics Vitros XT 7600, Raritan, NJ, USA). Lipids, glucose, and creatinine determinations were performed by standard methods (ADVIA 2400 Chemistry System, Siemens, Munich, Germany). The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

Statistical analysis

Quantitative data following a normal distribution are presented as mean ± standard deviation, and those with a not normal distribution are displayed as median (interquartile range). Qualitative variables are presented as percentages.

Univariable Cox regression was performed to analyse which variables were associated with the development of the outcomes. Then, multivariable regression analysis was carried out including those variables that achieved statistical significance at univariable analyses. Kaplan–Meier curves were traced showing the development of the different outcomes according to baseline FGF23 levels above the median or not. Cox univariable and multivariable analyses were repeated dividing the population between those with eGFR $<$60 mL/min/1.73 m^2 or not. The Youden Index was estimated to describe sensitivity and specificity values of the biomarkers.

Analyses were performed with SPSS 20.0 (SPSS Inc., New York) and were considered significant when *P* was lower than 0.05 (two-tailed).

Results

Characteristics of the population

The first table (Table 1) shows the baseline characteristics of the population. Age was 61.7 ± 12.2 years, and 77.1% were men. Twenty-two percent had diabetes, 57.1% hypertension, 60.1% dyslipidaemia, and 42.6% were smokers. Forty-nine per cent had a STEMI, and 51% had a NSTEACS. Eighty percent underwent percutaneous revascularization and 5% coronary artery by-pass graft, and 15% were not revascularized. A left ventricular ejection fraction (LVEF) $<$ 40% was present in 14.3%. Patients were well treated at discharge, with 95.5% receiving acetylsalicylic acid, 95.9% statins, and 83% beta-blockers. Only 14.1% had an eGFR $<$60 mL/min/1.73 m^2 (overall mean 78.5 ± 20.0 mL/min/1.73 m^2). Median time for blood extraction from admission was 4 (2–5) days.

Independent predictors of the primary outcome

Median follow-up was 5.44 (3.03–7.46) years. Two hundred ninety-four patients (24.7%) of the total population developed the primary outcome. Among them, 141 patients developed an ACS, 49 a cerebrovascular accident, 68 HF, and 115 died. Ten patients developed three different types of events, 59 had two different events, and the remaining patients developed a single event.

Table 1 Baseline data

Variable	(n = 1190)
Age (years)	61.67 ± 12.19
Age > 64 years	480 (40.3%)
Male sex	917 (77.1%)
Caucasian race	1151 (96.7%)
Diabetes	270 (22.7%)
Hypertension	680 (57.1%)
Body mass index (kg/m^2)	28.32 ± 4.32
Dyslipidaemia	715 (60.1%)
Smoking	507 (42.6%)
eGFR < 60 mL/min/1.73 m^2	168 (14.1%)
Coronary artery disease	240 (20.2%)
Peripheral artery disease	69 (5.8%)
Cerebrovascular accident	40 (3.4%)
Atrial fibrillation	28 (2.4%)
Number of affected vessels	1.45 ± 0.80
Laboratory results	
Troponin (ng/mL)	10.09 (0.78, 52.85)
eGFR (mL/min/1.73 m^2)	78.46 ± 19.99
LDL (mg/dL)	116.97 ± 36.85
Triglycerides (mg/dL)	131 (93.0, 182)
HDL (mg/dL)	40.25 ± 11.91
Calcidiol (ng/mL)	19.89 ± 9.86
PTH (pg/mL)	47.0 (36.0, 63.75)
FGF23 (RU/mL)	110.0 (84.0, 144.0)
Klotho (pg/mL)	629.49 ± 208.67
Phosphate (mg/dL)	3.26 (2.89, 3.63)
NT-proBNP (pg/mL)	405 (133.22, 1042.50)
Glycaemia (mg/dL)	104 (95.0, 124)
hs-CRP (ng/dL)	1.68 (0.72, 3.35)
Type of ACS and coronary angiographical findings	
ACS	
Non-STEACS	611 (51.3%)
STEMI	579 (48.7%)
LVEF < 40%	170 (14.3%)
Left main disease	40 (3.4%)
Complete revascularization	843 (70.8%)
Type of revascularization	
No revascularization	180 (15.1%)
Drug eluting stent	620 (52.1%)
Bare metal stent	299 (25.1%)
Balloon angioplasty	32 (2.7%)
Coronary artery bypass grafting	59 (5.0%)
Treatments at discharge	
ASA	1137 (95.5%)
P2Y12 blockers	1071 (90.0%)
Anticoagulant	80 (6.7%)
Statins	1141 (95.9%)
Ezetimibe	21 (1.8%)
Insulin	81 (6.8%)
Oral antidiabetic drugs	182 (15.3%)
ACEI/ARB	963 (80.9%)
Anti-aldosteronic drugs	95 (8.0%)
Beta-blockers	988 (83.0%)
Diuretics	182 (15.3%)

ACEI, angiotensin converting enzyme inhibitor; ACS, acute coronary syndrome; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HDL, high density lipoprotein; hs-CRP, high sensitivity C-reactive protein; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction; Non-STEACS, non-ST-elevation acute coronary syndrome; NT-ProBNP, N-terminal-pro-brain natriuretic peptide; PTH, parathormone; STEMI, ST-elevation myocardial infarction.

Univariable analysis showed that age, diabetes, hypertension, previous cardiovascular disorders, LVEF < 40% and treatment with anticoagulants, insulin and antidiabetic drugs, mineralcorticoid receptor antagonists or diuretics, among other factors, were positively associated with the development of the primary outcome. Other factors, such as eGFR, complete revascularization, and the use of acetylsalicylic acid, P2Y12 blockers, statins, and beta-blockers, showed an inverse association with the outcome (*Table* S1).

Table 2 Multivariable Cox regression analysis for the primary outcome of acute ischemic event, heart failure or death

Variable	HR (95% CI)	P
Coronary artery disease	1.73 (1.31, 2.29)	<0.001
Diuretic	1.52 (1.12, 2.06)	0.007
Age	1.03 (1.02, 1.04)	<0.001
FGF23[a]	1.18 (1.08, 1.29)	<0.001
Insulin	1.76 (1.18, 2.62)	0.006
Hypertension	1.63 (1.20, 2.21)	0.002
ACEI/ARB	0.62 (0.45, 0.85)	0.003
Cerebrovascular accident	1.74 (1.08, 2.82)	0.024
LVEF < 40%	1.46 (1.04, 2.07)	0.031
Beta-blockers	0.73 (0.54, 0.99)	0.041
Calcidiol[b]	0.86 (0.74, 1.00)	0.046

Proportional hazards test; P = 0.207. C statistic = 0.71.
ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; FGF23, fibroblast growth factor 23; HR, hazard ratio; LVEF, left ventricular ejection fraction.
[a]Every 100 units of increment.
[b]Every 10 units of increment.

Multivariable analysis (*Table 2*) showed that high FGF23, CAD or stroke, age, hypertension, LVEF < 40%, diuretics, and insulin were independently associated with this outcome (C statistic = 0.71). The best cut-off level was 154.5 RU/mL (sensitivity = 0.357, specificity 0.838; Youden index = 0.195). Calcidiol levels and the use of beta-blockers and modulators of renin-angiotensin system were inversely and independently related to the primary outcome. No interaction was found between FGF23 and calcidiol (P = 0.710).

Independent predictors of the incidence of acute ischaemic events

One hundred and eighty-four patients (15.5%) developed an acute ischaemic event. Six developed both an ACS and cerebrovascular accident, and the remaining subjects developed a single event. The univariable analysis is presented in Table S2.

Multivariable analysis (*Table 3*) showed that no biomarker had an independent predictive power for the outcome, while previous CAD and cerebrovascular accident, and hypertension had an independent positive association with it (C statistic = 0.67). On the other hand, STEMI as the index event, eGFR, and angiotensin-converting enzyme inhibitors/angiotensin II receptor blockers showed an inverse relationship.

Table 3 Multivariable Cox regression analysis for the secondary outcome of acute ischemic events

Variable	HR (95% CI)	*P*
Coronary artery disease	1.78 (1.25, 2.53)	0.001
eGFR[a]	0.89 (0.82, 0.97)	0.006
STEMI	0.69 (0.48, 0.99)	0.043
Hypertension	1.61 (1.10, 2.36)	0.014
ACEI/ARB	0.63 (0.43, 0.92)	0.016
Cerebrovascular accident	1.88 (1.01, 3.51)	0.047

Proportional hazards test; $P = 0.148$. C statistic = 0.67.
ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; STEMI, ST-elevation myocardial infarction.
[a]Every 10 units of increment.

Table 4 Multivariable Cox regression analysis for the secondary outcome of heart failure

Variable	HR (95% CI)	*P*
FGF23	1.45 (1.28, 1.65)	<0.001
PTH	1.06 (1.01, 1.12)	0.032
LVEF < 40%	3.84 (1.98, 7.45)	<0.001
Previous CVA	3.73 (1.84, 7.56)	<0.001
Age	1.08 (1.05, 1.11)	<0.001
Antidiabetic drugs	2.9 (1.61, 5.22)	<0.001
HDL	0.73 (0.57, 0.93)	0.012
Anti-aldosteronic drugs	2.44 (1.15, 5.15)	0.019

Proportional hazards test; $P = 0.042$. C statistic = 0.88.
CVA, Cerebrovascular Accident; FGF23, fibroblast growth factor 23; HDL, high density lipoprotein; HR, hazard ratio; LVEF, left ventricular ejection fraction; PTH, parathormone.

Independent predictors of the development of heart failure

Sixty-eight patients (5.7%) developed HF (Table S3). At multivariable analysis, high FGF23 resulted in an independent predictor of HF, as well as PTH, LVEF < 40%, previous cerebrovascular accident, age, and treatment with antidiabetic or anti-aldosteronic drugs (C statistic = 0.88). The best cut-off level was 112.5 RU/mL (sensitivity = 0.809, specificity 0.539; Youden index = 0.348). Cholesterol high-density lipoprotein was inversely related to the outcome (*Table 4*). No interactions were found among FGF23 and PTH ($P = 0.988$).

Independent predictors of death

One hundred and fifteen patients (9.7%) died. The cause of death was cardiovascular in 48 cases (4.0%), cancer in 18 (1.5%), infection in 14 (1.2%), unknown in 14 (1.2%), renal failure in 3 (0.3%), pancreatitis in 2 (0.2%), gastro-intestinal bleeding in 2 (0.2%), and other in 14 (1.2%). Univariable analysis is shown in Table S4. After multivariable analysis (*Table 5*), FGF23 and calcidiol, age, insulin, LVEF < 40%, acetylsalicylic acid, mineral receptor antagonists, and cholesterol low-density lipoprotein resulted independent predictors of death, being the latter two protective (C statistic = 0.82). The best cut-off level for this outcome was 167.5 RU/mL (sensitivity = 0.426, specificity 0.854; Youden index = 0.280). No interactions were found between FGF23 and calcidiol ($P = 0.492$).

Table 5 Multivariable Cox regression analysis for the secondary outcome of death

Variable	HR (95% CI)	*P*
Age	1.07 (1.05, 1.10)	<0.001
Insulin	2.85 (1.57, 5.16)	0.001
LVEF < 40%	2.00 (1.12, 3.57)	0.02
FGF23[a]	1.21 (1.07, 1.37)	0.002
ASA	0.41 (0.21, 0.79)	0.008
LDL	0.92 (0.86, 0.98)	0.011
Calcidiol[b]	0.72 (0.54, 0.97)	0.028
Anti-aldosteronic drugs	1.99 (1.01, 3.90)	0.046

Proportional hazards test; $P = 0.543$. C statistic = 0.82.
ASA, acetylsalicylic acid; CI, confidence interval; FGF23, fibroblast growth factor 23; HR, hazard ratio; LDL, low density lipoprotein; LVEF, left ventricular ejection fraction.
[a]Every 100 units of increment.
[b]Every 10 units of increment.

Prognostic power of FGF23 plasma levels above the median

Kaplan–Meier curves showed highly significant differences in patients with FGF23 levels above the median (110 RU/mL) about the primary outcome, HF, and death (*Figure 1*). We also performed Kaplan–Meier curves by dividing the population into two groups according to the FGF23 cut-off value of 92.8 RU/mL, which was found in the SOLID-TIMI trial to be the one that separated quartile 4 from quartiles 1–3 in that study.[6] The results were not more evident than using that median FGF23 cut-off. In fact, this threshold value represented the percentile 35th in our population. In our study, blood was withdrawn at discharge of the index event, whereas in the SOLID-TIMI trial, it was taken about 15 days after the index event. Thus, we hypothesized that FGF23 levels could decrease after an ACS, and we confirmed it in our series (*Table 6*).

Homogeneity of the results obtained across different population subgroups for the studied outcomes

No significant differences were shown when the incidence of the primary outcome, HF, and death was assessed according to FGF23 levels across subgroups (*Figure 2*).

Figure 1 Kaplan–Meier curves showing the incidence of cardiovascular events once divided the population according to median FGF23 values with the cut-off values of our study (110 RU/mL) and the cut-off values of the SOLID-TIMI52 trial (92.8 RU/mL).[11]

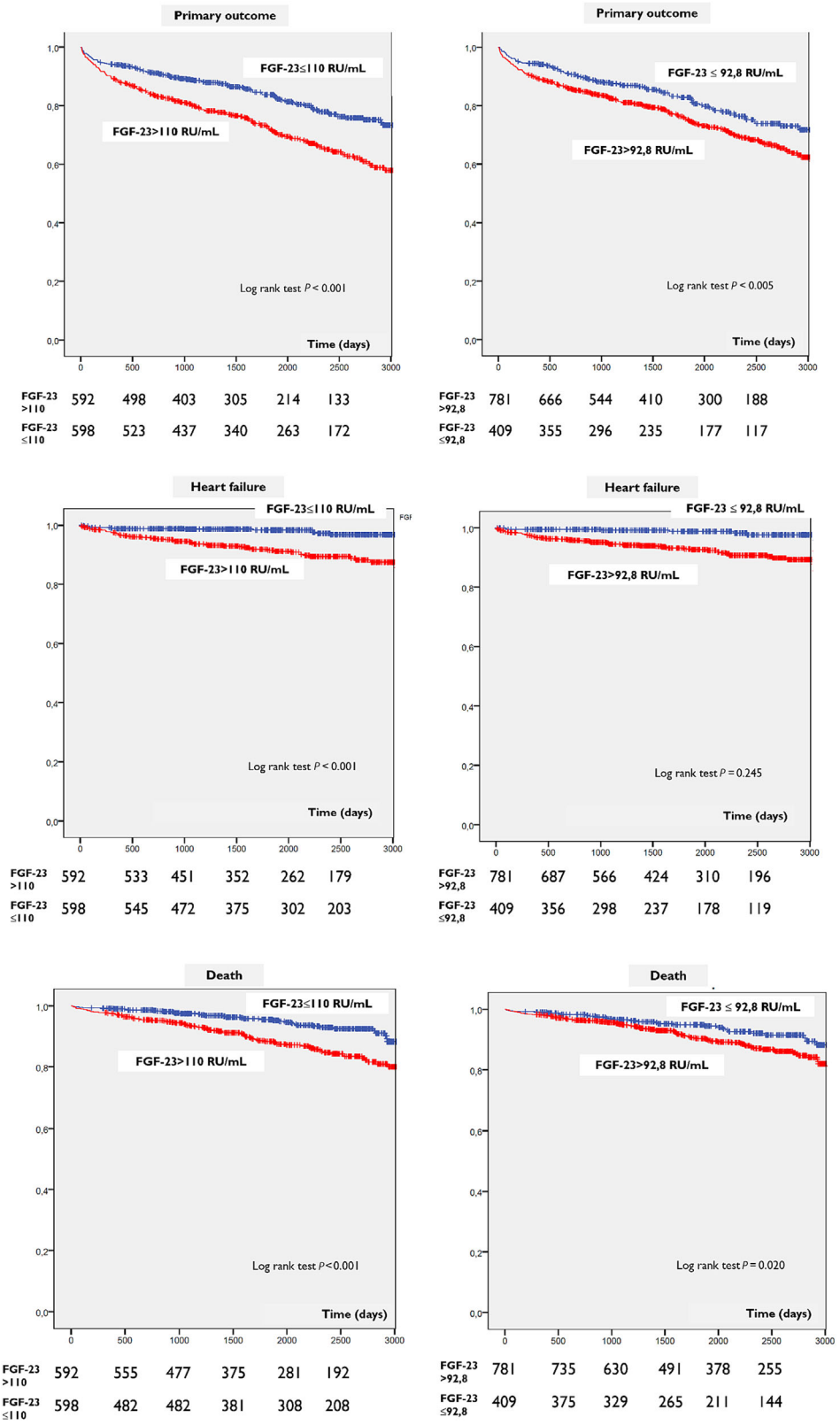

Table 6 Comparison of plasma levels of mineral metabolism components at discharge and 6 months later

Variable	Visit 0	Visit 6 months	Difference	*P*
Calcidiol (ng/mL)	19.0 (13.5, 25.3)	19.3 (14.3, 25.5)	0.20 (−5.57, 5.59)	0.486
PTH (pg/mL)	46.1 (36.0, 62.0)	58.0 (44.5, 75.3)	10.5 (−1.5, 23.4)	<0.001
FGF23 (RU/mL)	110 (84, 145)	79.1 (59, 103)	−26.9 (−54.60, −3.85)	<0.001
Klotho (pg/mL)	589 (486, 734)	568 (472, 690)	−18.6 (−125.2, 74.2)	<0.001
Phosphate (mg/dL)	3.28 (2.90, 3.64)	3.20 (2.80, 3.50)	−0.16 (−0.55, 0.26)	<0.001
NT-proBNP (pg/mL)	393 (128, 992)	176 (91, 387)	−162 (−577.5, 17.2)	<0.001

Median is shown. Quartiles are shown in brackets.
FGF23, fibroblast growth factor 23; NT-proBNP, N-terminal-pro-brain natriuretic peptide; PTH, parathormone.

Figure 2 Forrest plots showing the prognostic value of FGF23 levels above the median for the primary and secondary outcomes across different population subgroups.

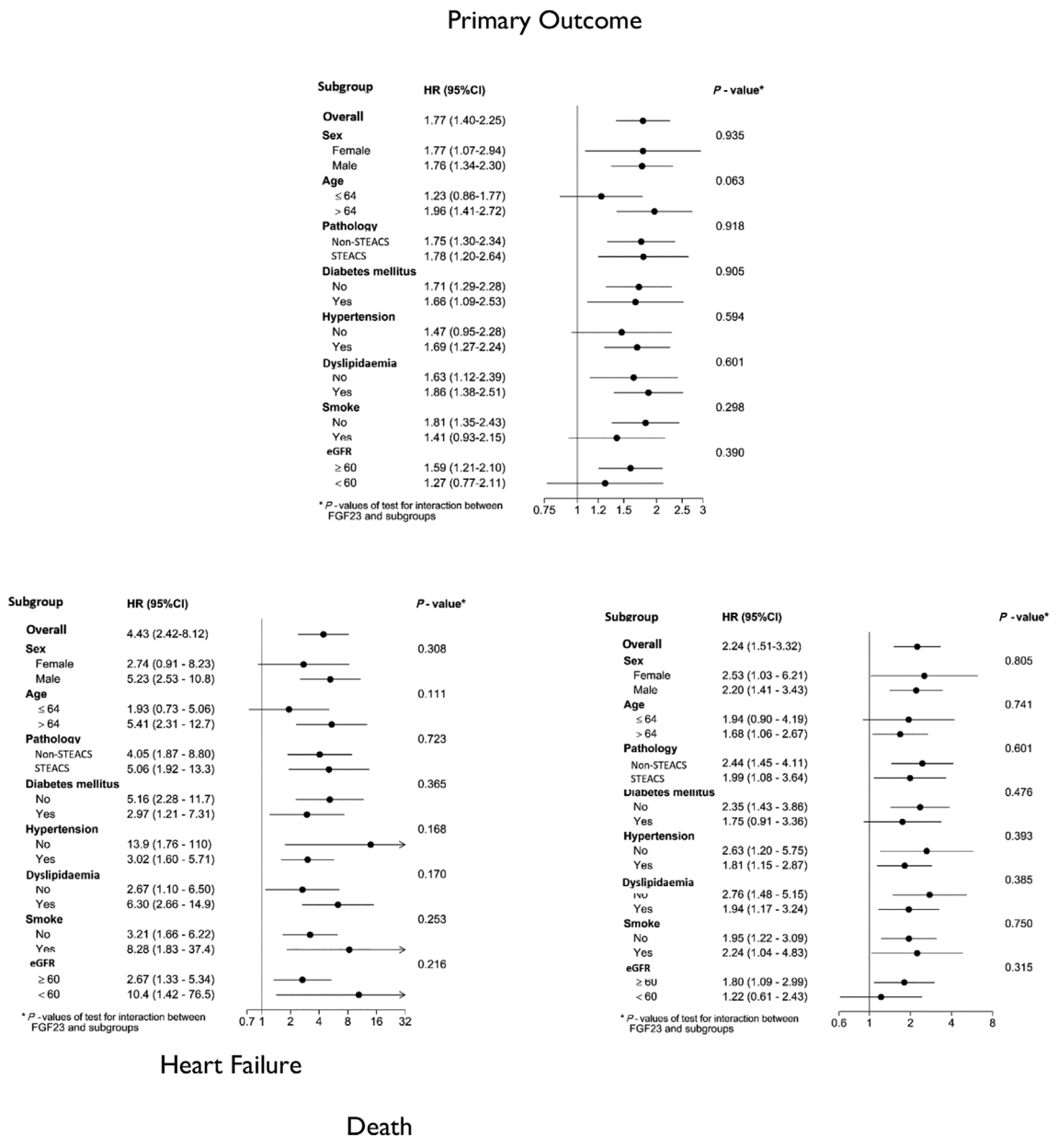

Table 7 Multivariable Cox regression analysis for the primary outcome according to eGFR

Variable	HR (95% CI)	*P*
(a) eGFR ≥ 60 mL/min/1.73 m^2		
Age	1.02 (1.01–1.04)	0.002
FGF23[a]	1.16 (1.05–1.30)	0.005
Diuretics	1.67 (1.12–2.48)	0.012
Hypertension	1.49 (1.07–2.09)	0.02
Beta-blockers	0.67 (0.47–0.96)	0.031
Insulin	1.80 (1.01–3.21)	0.044
Calcidiol[b]	0.82 (0.68–0.99)	0.042
(b) eGFR < 60 mL/min/1.73 m^2		
eGFR[a]	0.74 (0.59–0.93)	0.009
Anticoagulant	3.14 (1.69–5.84)	<0.001
LDL[b]	0.89 (0.84–0.95)	0.001
Age	1.03 (1.00–1.06)	0.030
Previous CVA	2.16 (1.15–4.06)	0.017

For (a), proportional hazards test; $P = 0.251$. C statistic = 0.67. For (b), proportional hazards test; $P = 0.095$. C statistic = 0.70.
CI, confidence interval; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HR, hazard ratio; LDL, low density lipoprotein.
[a]Every 100 units of increment.
[b]Every 10 units of increment.

Independent predictive power of fibroblast growth factor 23 for the primary outcome according to renal function

We assessed eGFR with the CKD-EPI equation.[23] Two hundred patients (18.9%) had an eGFR < 60 mL/min/1.73 m^2. Among them, no components of MM were independently associated with the primary outcome. However, considering the group of eGFR ≥ 60 mL/min/1.73 m^2, FGF23 was positively and independently associated with the primary outcome, while calcidiol was inversely related to it (*Table* 7).

Discussion

In our study, elevated concentrations of FGF23 were a strong independent predictor of the primary outcome despite adjusting for a large set of variables, including cardiovascular risk factors, previous cardiovascular history, data from the index events, and medical therapy. This was due to its ability to predict HF and death. The large number of variables included in the multivariate test support the idea of FGF23 as an independent predictor of this outcome instead of a surrogate marker for sicker patients. Of interest, low calcidiol levels also added independent predictive value for both, the development of the primary outcome and of death, while PTH provided significant independent prognostic ability on the development of HF. Furthermore, after adjusting for other biomarkers, such as NT-proBNP, the prognostic value of FGF23 was not altered. Moreover, NT-proBNP and hs-CRP plasma levels did not provide additional prognostic value and were not positive in the multivariable model. Although klotho plasma levels tended to be associated with a reduced incidence of all studied outcomes, this association did not reach statistical significance even at univariable analysis.

The results on FGF23 are consistent with previous studies in the field, with the exception that they did not include a complete assessment of the MM status. However, some differences should be noted. In a sample of 125 patients with acute HF, high FGF23 levels at discharge predicted a higher incidence of HF or death at 1 year of follow-up.[24] Also, in 1099 patients with acute myocardial infarction, the prognostic value of 175 biomarkers was explored after a median follow-up of 6.6 years.[25] FGF23 was among those biomarkers predicting the outcome of total death, recurrent acute myocardial infarction, or HF hospitalization, although the number of clinical variables employed for adjustment was lower than in our study. A meta-analysis including more than 135 000 participants from the general population showed that FGF23 is an independent predictor of the development of HF, stroke, and acute myocardial infarction.[26] In the community-based PREVEND (Prevention of Renal and Vascular End-Stage Disease) study, a higher FGF23 level was associated with the development of HF with reduced ejection fraction but not with the appearance of HF with preserved ejection fraction during follow-up.[27] Finally, in a secondary analysis from the Stabilization of Plaques Using Darapladib-Thrombolysis in Myocardial Infarction 52 (SOLID-TIMI 52) trial including 4947 patients with ACS, FGF23 was found to be an independent predictor of cardiovascular death or hospitalization by HF.[28] However, some differences from our work should be noted. Firstly, in our study, we analysed all components of MM and not only FGF23 levels. Secondly, in the multivariable analysis, we considered ongoing treatments at discharge, which may have an additional role in the prediction of recurrent events of interest. Finally, we performed FGF23 assessment at discharge after the index event, while in the SOLID-TIMI52, FGF23 determination was performed in the 30 days following the index event. This is more complicated than just assessing FGF23 during ACS admission. Interestingly, although they used the FGF23 concentration that separated quartile 4 from 1–3 as a cut-off, these values were clearly lower than the median from our study (92.8 mg/dL vs. 110 mg/dL), and when we tried to use this cut-off in our population, the predictive value of FGF23 did not improve. We explained this difference as a result of the different timing of FGF23 measurement, and we confirmed in our own population that FGF23 levels decreased during the 6 months following the ACS. Therefore, it might not be the same to assess FGF23 levels during ACS than assessing it in the following weeks.

Role of fibroblast growth factor 23 in cardiovascular disease

Abnormalities of MM are not exclusive to CKD patients, as we have found previously that almost 20% of patients with sta-

ble CAD and eGFR $\geq$ 90 mL/min/1.73 m^2 have increased plasmatic concentrations of FGF23 and PTH, and more than 60% of them have low calcidiol levels.[19] In line with this, other situations different to CKD, such systemic inflammatory processes, diabetes, ACS, and shock, show high FGF23 levels.[29] On this regard, FGF23 seems to be involved in vascular and, specifically, myocardial damage,[15,30] irrespective of the coexistence of traditional cardiovascular risks factors. Thus, it has been linked to many cardiac diseases, mainly HF, and acute[31–33] and chronic[16,20,33,34] CAD, through causing left ventricular hypertrophy,[15] cardiac fibrosis, and ventricular remodelling.[29,35] These effects have been called the paracrine expression of FGF23 and are responsible for the cardiac adaptation to its imbalance.[36] Fibroblast growth factor 23 is over-expressed in a mouse model of right ventricular pressure overload mainly when there is fibrosis accompanying ventricular hypertrophy, while its receptor FGFR1 is over-expressed in the fibroblasts.[37] In this way, FGF23 could act as a paracrine factor produced by cardiomyocytes, thus promoting fibrosis in adjacent fibroblasts. In addition, the pro-fibrotic effect of FGF23 on fibroblasts in culture shows a synergy with transforming growth factor β1 (TGF-β1),[37] a well-known mediator of fibrosis. Animal models have also demonstrated that not only high FGF23 concentrations but also the length of exposure to them are critical factors regarding cardiac adaptation.[31] Furthermore, while the heart suffers because of those MM changes, it is not only a passive spectator. On the contrary, severe cardiac conditions, such as cardiogenic shock, also increase the production of FGF23, creating a vicious circle of adverse adaptations.[38] No safe levels of FGF23 have been established, nor have any potential benefits of its blockade, as animal models have linked the latter to aortic calcification and higher mortality.[38]

Predictive power of fibroblast growth factor 23 across subgroups

Given the strong relationship between MM and renal function, it could be possible that the results observed were due to the prognostic power of FGF23 in patients with CKD. However, when multivariable regression analysis by subgroups according to renal function was performed, FGF23 was a strong, independent predictor of the primary outcome in patients with eGFR $\geq$ 60 mL/min/1.73 m^2. In patients with lower eGFR, it did not retain this predictive power, although this could be due to the low sample size of this subgroup. Similarly, when we performed subgroup analysis, no differences were evident for the predictive power of FGF23 according to sex, age, and the presence of hypertension or diabetes among other factors. These findings are similar to those reported in the sub-analysis of the SOLID-TIMI 52 trial.[28]

Relevance of a complete assessment of mineral metabolism for cardiovascular risk prediction

In addition to the consistent power of FGF23 to predict cardiovascular events, in our series, low calcidiol levels added independent predictive value for the primary outcome and death, while high PTH did so for HF. Furthermore, in a previous paper, we analysed the prognostic value of MM components in 964 patients from this population, 6 months after the ACS, in other words, when the patients had stable CAD.[11] At this point, PTH but not FGF23 turned out to be an independent prognostic marker of acute ischaemic events, HF, or death. Although the ultimate reason for this finding is still unknown, we speculate that it may be related to the changes in the MM that we observed the six-month period after the ACS. In this regard, in addition to the decrease in FGF23 plasma levels, there is an increase of PTH levels and a decrease of soluble klotho and phosphate levels. These data, as well as the evidence of the interaction between the prognostic value of calcidiol and FGF23 that we had demonstrated previously,[20] suggest that a complete assessment of MM may be necessary to answer the outstanding questions in this area in the future.

Limitations

The design of the study required the collection of plasma for analysis at discharge no later than 6 days after admission, to achieve homogeneous results. This lead to the exclusion of 10.9% of ACS patients who did not meet this condition and that fact may explain the low number of cases with LVEF $<$ 40% that were included. Therefore, these results should not be extrapolated to populations with a high percentage of patients with moderate or severe LV systolic dysfunction.

Conclusions

Among a population of patients with ACS with average eGFR, FGF23 is a strong independent predictor of HF and death, along with other components of MM, even after adjustment of a large set of variables, including NT-proBNP plasma levels. This effect was homogeneous across different population subgroups and not limited to patients with CKD.

Clinical perspectives

- Mineral metabolism has been linked to deleterious adaptations on the cardiovascular system, not only in patients with CKD.

- We explore for the first time the potential predictive value of a myriad of mineral metabolism components in a series of patients after an acute coronary syndrome.
- Our results show they independently predict adverse outcomes such us death and heart failure.
- Also, they enhance the importance of assessing the whole pack of mineral metabolism components and to do it in the acute phase.
- Finally, no other biomarkers such as hs-CRP and NT-proBNP resulted independent prognostic markers.

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Conflict of interest

No conflicts of interest have to be declared.

Supporting information

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

Data S1. Supporting Information

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