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ORIGINAL RESEARCH ARTICLE

Parathormone levels add prognostic ability to N-terminal pro-brain natriuretic peptide in stable coronary patients

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Abstract

Aims There are controversial data on the ability of the components of mineral metabolism (vitamin D, phosphate, parathormone [PTH], fibroblast growth factor-23 [FGF23], and klotho) to predict cardiovascular events. In addition, it is unknown whether they add any prognostic value to other well-known biomarkers.

Methods and results In 969 stable coronary patients, we determined plasma levels of all the aforementioned components of mineral metabolism with a complete set of clinical and biochemical variables, including N-terminal pro-brain natriuretic peptide (NT-proBNP), high-sensitivity troponin I (hs-TnI), and high-sensitivity C-reactive protein. Secondary outcomes were ischaemic events (any acute coronary syndrome, stroke, or transient ischaemic attack) and heart failure or death. The primary outcome was a composite of the secondary outcomes. Median follow-up was 5.39 years. Age was 60 (52–72) years. Median glomerular filtration rate was 80.4 (65.3–93.1) mL/min/1.73 m^2. One-hundred and eighty-five patients developed the primary outcome. FGF23, PTH, hs-TnI, and NT-proBNP were directly related with the primary outcome on univariate Cox analysis, while Klotho and calcidiol were inversely related. On multivariate analysis, only PTH (HR 1.058 [CI 1.021–1.097]; $P = 0.002$) and NT-proBNP (HR 1.020 [CI 1.012–1.028]; $P < 0.001$) were independent predictors of the primary outcome but also for the secondary outcome of heart failure or death (HR 1.066 [CI 1.016–1.119]; $P = 0.009$ and HR 1.024 [CI 1.014–1.034]; $P < 0.001$, respectively). PTH was the only biomarker that predicted ischaemic events (HR 1.052 [1.010–1.096]; $P = 0.016$). Patients were divided in two subgroups according to FGF23 plasma levels. PTH retained its prognostic value only in patients with FGF23 levels above the median (>85.5 RU/mL) ($P < 0.001$) but not in patients with low FGF23 levels ($P = 0.551$). There was a significant interaction between FGF23 and PTH ($P = 0.002$). However, there was no significant interaction between PTH and both klotho and calcidiol levels.

Conclusions Parathormone is an independent predictor of cardiovascular events in coronary patients, adding complimentary prognostic information to NT-proBNP plasma levels. This predictive value is restricted to patients with high FGF23 plasma levels. This should be considered in the design of future studies in this field.

Keywords Parathormone; Fibroblast growth factor-23; Coronary artery disease; Mineral metabolism

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Introduction

In the last decade, vitamin D has raised attention due to its relation with cardiovascular disease development, as a deficiency of vitamin D is associated with an increase in the incidence of cardiovascular adverse events.[1,2] However, vitamin D is part of a system known as mineral metabolism, which encompasses several other components, such as fibroblast growth factor 23 (FGF23), parathormone (PTH), and phosphate, that may be also related to the incidence of cardiovascular disease.

Fibroblast growth factor 23 is a phosphaturic hormone that helps the diseased kidney eliminate phosphate and decreases excessive vitamin D levels.[3] High FGF23 plasma levels have been associated with increased mortality, heart failure, and left ventricular (LV) hypertrophy.[4,5] Similarly, increased PTH plasma levels are related to hypertension, LV hypertrophy, and increased cardiovascular events.[6–9] More recently, the soluble form of klotho, the co-receptor of FGF23, has been associated with antiaging and exerts protective cardio-renal effects.[10,11]

Despite this body of evidence, there are no studies exploring if the aforementioned components of mineral metabolism add prognostic value to other well-established biomarkers, such as N-terminal pro-brain natriuretic peptide (NT-proBNP) and high-sensitivity troponin (hs-TnI), in patients with stable coronary artery disease. Thus, the purpose of this study is to determine whether the analysis of the components of mineral metabolism in this setting adds prognostic information to NT-proBNP and hs-TnI plasma levels.

Methods

Patients

Nine hundred and sixty-nine patients with stable coronary artery disease, who had suffered an acute coronary syndrome 6–12 months before were included in this study. These patients were part of the BACS & BAMI (Biomarkers in Acute Coronary Syndrome & Biomarkers in Acute Myocardial Infarction) studies, carried out in five hospitals in Madrid. Inclusion and exclusion criteria have been defined previously.[12] Between July 2006 and June 2014, 2740 patients were discharged from the study hospitals with a diagnosis of NSTEACS or STEMI (Supporting Information, *Figure S1*); 1483 patients were excluded due to the following: 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 (11.9%), concomitant mental disorders (4.4%), 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%). Finally, 1257 patients were included. On admission, clinical variables were recorded, and plasma was withdrawn for analysis.

Six to 12 months after discharge, patients were seen again and clinical variables and a second plasma extraction was performed. The present paper is a sub-study of BACS & BAMI studies and reports data from the clinical and analytic findings obtained at the time of this second plasma extraction, relating them to subsequent follow-up. Of the 1257 patients included in the acute phase, 284 did not go to the hospital for the second plasma extraction and four were excluded because they developed a cancer. Thus, 969 patients had adequate plasma samples for the present analysis. Plasma extraction and baseline visits took place between January 2007 and December 2014. Last follow-up visits were carried out on June 2016.

Ethics statement

The research protocol conforms to 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, Hospital Fundación Alcorcón, Hospital de Fuenlabrada, Hospital Universitario Puerta de Hierro Majadahonda, and Hospital Universitario de Móstoles. All patients signed informed consent documents.

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 hospital. 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 (NSTEACS, STEMI, stroke, and transient ischaemic attack) plus heart failure and all-cause mortality. Secondary outcomes were ischaemic events and the composite of heart failure 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 two adjacent leads in the electrocardiogram without response to nitroglycerin, and troponin elevation. Stroke was defined as rapid onset of a neurologic deficit attributable to a focal vascular cause lasting more than

24 h or supported by new cerebral ischaemic lesions at imaging studies. A transient ischaemic attack was defined as a transient stroke with signs and symptoms resolving before 24 h without cerebral acute ischaemic lesions at imaging techniques. Heart failure was a clinical diagnosis made in accordance to practice guidelines.[13] Events were adjudicated by at least two investigators of the study, along with a neurologist for cerebrovascular events. Although all events were recorded for each case, patients were excluded from the Cox regression analysis after the first event. Then, although the total number of events is also described, patients that had more than one event were computed only once for these analyses.

Biomarker and analytical studies

Plasma determinations were performed at the laboratory of Nephrology at the Gómez-Ulla hospital and at the Biochemistry Laboratory at Fundación Jiménez Díaz. The investigators who performed the laboratory studies were unaware of clinical data. Plasma calcidiol levels were quantified by chemiluminescent immunoassay (CLIA) on the LIAISON XL analyser (LIAISON 25OH-Vitamin D total Assay DiaSorin, Saluggia, Italy), FGF23 was measured by an enzyme-linked immunosorbent assay which recognizes epitopes within the carboxyl-terminal portion of FGF-23 (Human FGF23, C-Term, Immutopics Inc, San Clemente, CA), klotho levels by ELISA (Human soluble alpha klotho assay kit, Immuno-Biological Laboratories Co., Japan), intact parathormone was analysed by a second-generation automated chemiluminescent method (Elecsys 2010 platform, Roche Diagnostics, Mannheim, Germany), phosphate was determined by an enzymatic method (Integra 400 analyser, Roche Diagnostics, Mannheim, Germany), and high-sensitivity C-reactive (hs-CRP) protein was assessed by latex-enhanced immunoturbidimetry (ADVIA 2400 Chemistry System, Siemens, Germany). Lipids, glucose, and creatinine determinations were performed by standard methods (ADVIA 2400 Chemistry System, Siemens, Germany). The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.

Statistical analysis

Quantitative data following a normal distribution are presented as mean ± standard deviation (SD), those not normally distributed are displayed as median (interquartile range), and qualitative variables are presented as percentages. Furthermore, correlations between quantitative variables were assessed with the Spearman's rho coefficient (*r*).

Differences in baseline data of patients meeting the primary outcome as compared with those remaining stable were assessed using χ^2 or Fisher exact test for qualitative data. For quantitative variables, a Student's *t*-test was performed for those following a normal distribution, and the Mann–Whitney test was used in those not normally distributed.

Univariate Cox regression was performed to analyse which variables were associated with the development of the different outcomes. Then, multivariate regression analysis was carried out including those variables that achieved statistical significance at univariate analyses. Finally, Kaplan–Meier curves were developed to explore potential changes in the predictive value of PTH according to the status of other mineral metabolism biomarkers. For this purpose, patients were divided in two subgroups of high vs. low levels of mineral metabolism markers. FGF-23 >85.5 RU/mL[14,15] and PTH >65 ng/L[6] were considered high levels, and calcidiol ≤20 mmol/L[15] were considered low levels. In the case of klotho, given the lack of previous reference values, we used the median of our population as a cut-off. Analyses were performed with SPSS 19.0 (SPSS Inc., New York) and were considered significant when *P* was lower than 0.05 (two-tailed).

Results

Patients

A total of 969 patients were included, but five patients were lost during follow up, so finally there were 964 patients for the analysis. Baseline characteristics are detailed in *Table 1* (full data in the Supporting Information, *Table S1*). Median age was 60 years and median eGFR 80.4 (65.3–93.1) mL/min/1.73 m^2, with 82.0% of the population displaying an eGFR ≥60 mL/min/1.73 m^2. Most patients were male (76.2%). Left ventricular ejection fraction was lower than 40% in 7%, and 11.6% of the patients had a history of previous heart failure. Time elapsed since the previous acute coronary syndrome was 6.5 (6.2–7.6) months.

Abnormalities of mineral metabolism

Abnormal levels of the components of mineral metabolism were frequently found. Thus, in the global cohort, 87.6% had low or suboptimal calcidiol levels (≤30 ng/mL). In addition, elevated levels of FGF23 (>85.5 RU/mL) and PTH (>PTH 65 pg/mL) were observed in 40.9% and 37.9% of patients, respectively, while only 0.9% had elevated phosphate (>4.5 mg/dL). We also studied the differences in the levels of the components of mineral metabolism dividing the patients according to whether they had eGFR <60 mL/min/1.73 m^2 or higher. Patients with eGFR <60 mL/min/1.73 m^2 had higher levels of phosphate, FGF23 and PTH, and

Table 1 Baseline characteristics

Variable	
Age (year)	60 (52–72)
Sex, male/female (%)	76.2/23.8
Smoker (%)	13.9
Hypertension (%)	64.2
Diabetes (%)	24.1
Dyslipidaemia (%)	60.3
LVEF <40 (%)	7
Prior heart failure (%)	11.6
Treatment	
Aspirin (%)	93.6
P2Y12 antagonist (%)	75.4
Statin (%)	94.8
ACEI (%)	62.6
ARB (%)	15.4
Aldosterone antagonist (%)	6.7
Beta-blocker (%)	79.0
Diuretic (%)	18.8
Vitamin D (%)	1.35
Last acute coronary syndrome	
STEMI/NSTEACS (%)	49.6/50.4
Number of vessel disease	1.4 ± 0.79
Revascularization method (%)	
• No revascularization	14.7
• PCI	80.4
• CABG	4.9
Incomplete revascularization (%)	29.8
Analytics	
Glucose (mmol/L)	5.61 (5.05–6.38)
LDL (mmol/L)	1.99 (1.66–2.38)
eGFR	80.4 (65.3–93.1)
HsCRP (mg/L)	1.15 (0.37–3.11)
NT-proBNP (ng/L)	176.5 (90.7–393.25)
HsTroponin I (μg/L)	0.003 (0.000–0.010)
Phosphate (mmol/L)	3.2 (2.8–3.5)
Calcidiol (mmol/L)	12.9 (14.2–25.3)
Calcidiol categories (%)	
• ≤10 ng/mL	10.3
• 10.01–20 ng/mL	43.9
• 20.01–30 ng/mL	33.4
• >30 ng/mL	12.4
FGF23 (RU/mL)	78.5 (59.1–102.6)
Klotho (pg/mL)	568.9 (469.7–690.6)
PTH (ng/L)	57.9 (44.3–75.3)

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMS, bare metal stent; CABG, coronary artery bypass graft; DES, drug eluting stent; eGFR, estimated glomerular filtration rate; FGF23, fibroblast growth factor 23; HDL, High-density lipoprotein; Hs, high-sensitivity; HsCRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein; LVEF, left ventricle ejection fraction; NSTEACS, non-ST elevation acute coronary syndrome; NT-proBNP, N-terminal pro-brain natriuretic peptide; PTH, parathormone; STEMI, ST-elevation myocardial infarction.

lower klotho plasma levels, while there were no significant differences in calcidiol levels (Supporting Information, *Table S2*).

Clinical events

During a median follow-up of 5.39 years (2.81–6.92), 185 patients developed the primary end point with a total of 278 events. There were 108 acute coronary syndromes (44 unstable angina, 48 NSTEMI, and 16 STEMI), 36 cerebrovascular events (18 stroke and 18 TIA), 59 episodes of heart failure, and 75 deaths. Thirty-eight patients presented two events, 20 presented three events, and 5 patients four events.

Regarding the 75 deaths observed during follow-up, 28 were of cardiovascular origin, 15 were due to cancer, 9 to infection, 3 to renal failure, 3 to advanced cognitive disorders, 2 to pancreatitis, 2 to gastrointestinal bleeding, 2 to exacerbation of pulmonary disease, 3 were due to other causes, and 8 were of unknown origin.

Finally, 116 patients developed an acute ischaemic event, and 96 patients developed an episode of death or heart failure.

Prognostic value of the components of mineral metabolism

At the univariate analysis, there were many variables associated with the development of the primary and secondary outcomes (Supporting Information, *Table S3*). FGF23, PTH, hs-TnI, and NT-proBNP were directly related with the primary outcome, while klotho and calcidiol were inversely related, and phosphate did not show statistical significance. To identify the independent predictors of adverse outcomes, we performed multivariate analysis including all the statistically significant variables, sex, and age. For the primary outcome, we used two different models; the first one did not include NT-proBNP and hs-TnI in the analysis while the second did. Both FGF23 and PTH showed statistical association with the primary outcome in the first model. However, FGF23 lost significance in the second model, while PTH, hs-TnI, and NT-proBNP were independent predictors of the development of this outcome (*Table 2*).

The results of the univariate test were similar for the secondary outcome of heart failure and death, except that in this case, phosphate also showed a significant direct relationship (Supporting Information, *Table S3*). In the multivariate test, only PTH and NT-proBNP were independent predictors of this outcome (*Table 3*).

Finally, regarding the development of ischaemic events in the univariate test, PTH and FGF23 were directly associated with the incidence of this outcome, and calcidiol showed an inverse association. Neither klotho, phosphate, hs-TnI nor NT-proBNP showed any statistical association with this endpoint (Supporting Information, *Table S3*). At multivariate analysis, PTH was the only biomarker retaining independent statistical significance (*Table 4*).

The incidence of the primary outcome and the secondary of heart failure or death was higher for patients with PTH levels in the fourth quartile, while there was not significant difference in the secondary outcome of ischaemic events (*Figure 1*).

Table 2 Multivariate analysis for the primary outcome

Variable	HR	Confidence interval	*P*
Model 1			
Age	1.034	(1.020–1.047)	**<0.001**
Prior heart failure	1.603	(1.101–2.235)	**0.014**
Statin	0.367	(0.228–0.590)	**<0.001**
Insulin	1.992	(1.231–3.224)	**0.005**
Nitrates	1.678	(1.180–2.387)	**0.004**
ARB	1.487	(1.041–2.124)	**0.029**
Proton pump inhibitors	1.857	(1.290–2.673)	**0.001**
NonHDL	1.005	(1.000–1.009)	0.053
FGF23[a]	1.076	(1.010-1.147)	**0.024**
PTH[b]	1.070	(1.032-1.109)	**<0.001**
Model 2			
Age	1.031	(1.018–1.045)	**<0.001**
Statin	0.328	(0.219–0.553)	**<0.001**
Insulin	2.025	(1.264–3.243)	**0.003**
Nitrates	1.539	(1.069–2.214)	**0.020**
ARB	1.535	(1.081–2.181)	**0.017**
Aldosterone antagonist	1.571	(0.956–2.579)	0.074
Proton pump inhibitors	2.029	(1.408–2.924)	**<0.001**
STEMI	0.726	(0.517–1.018)	0.063
NonHDL	1.004	(1.000–1.009)	0.071
PTH[b]	1.058	(1.021-1.097)	**0.002**
NT-proBNP[a]	1.020	(1.012-1.028)	**<0.001**

Model 1: includes sex, age, and all variables that showed statistical significance at univariate analysis, except hs-troponin I and NT-proBNP. Model 2: Variables included in model 1 plus hs-troponin and NT-proBNP.
ARB, angiotensin II receptor blocker. FGF23, fibroblast growth factor 23. HDL, high-density lipoprotein. NT-proBNP, N-terminal pro-brain natriuretic peptide. PTH, parathormone. STEMI, ST-elevation myocardial infarction.
[a]HR per every 100-unit increase.
[b]HR per every 10-unit increase.

Table 3 Multivariate analysis for secondary outcome of HF and death

Variable	HR	Confidence interval	*P*
Age	1.072	(1.050–1.097)	**<0.001**
Prior heart failure	1.861	(1.115–3.108)	**0.017**
LVEF<40	1.728	(0.990–3.014)	0.054
Statin	0.522	(0.268–1.018)	0.056
Insulin	2.265	(1.179–4.353)	**0.014**
Beta-blocker	0.545	(0.348–0.853)	**0.008**
Aldosterone antagonist	1.703	(0.935–3.102)	0.082
Proton pump inhibitors	1.699	(0.995–2.900)	0.052
Phosphate	1.403	(0.957–2.059)	0.083
PTH[a]	1.066	(1.016-1.119)	**0.009**
NT-proBNP[b]	1.024	(1.014-1.034)	**<0.001**

LVEF, left ventricle ejection fraction; NT-proBNP, N-terminal pro-brain natriuretic peptide; PTH, parathormone.
[a]HR per every 10-unit increase.
[b]HR per every 100-unit increase.

Influence of other mineral metabolism markers in the predictive value of parathormone

To test the effect of other mineral metabolism markers on the predictive value of PTH on the primary outcome we divided the population in subgroups according to FGF23, klotho, and calcidiol plasma levels. While for FGF-23, PTH, and calcidiol, we used previously established cut-off levels, as described in Methods section, for klotho, we used the median (658.85 pg/mL) as a cut-off point. PTH showed an excellent prognostic value in patients with high FGF23, and no prognostic value in patients with normal FGF23 (*Figure 2*). Similarly, PTH was able to predict the primary outcome in patients with calcidiol levels ≤20, but not in the opposite condition, although the differences were less marked than those found in the FGF23 subgroups (*Figure 2*). In this regard, there was a significant interaction between PTH and FGF23 (P = 0.013), but not between PTH and klotho (P = 0.303) or calcidiol (P = 0.915) (Supporting Information, *Table S4*). We tested multicollinearity using the variance inflation factor (VIF) that yielded values between 1 and 2 for all predictors, suggesting that multicollinearity was not present.

Table 4 Multivariate analysis for secondary outcome of ischaemic events

Variable	HR	Confidence interval	*P*
Statin	0.392	(0.222–0.692)	**0.001**
Insulin	2.432	(1.403–4.215)	**0.002**
ACEI	0.674	(0.463–0.981)	**0.039**
Proton pump inhibitor	2.173	(1.370–3.638)	**0.001**
Dihydropyridine	1.509	(0.970–2.347)	0.068
STEMI	1.259	(1.020–1.553)	**0.032**
Number vessel disease	1.311	(1.062–1.617)	**0.012**
NonHDL	1.008	(1.002–1.013)	**0.005**
PTH[a]	1.052	(1.010-1.096)	**0.016**

ACEI, angiotensin converting enzyme inhibitor; HDL, high-density lipoprotein; PTH, parathormone; STEMI, ST-elevation myocardial infarction.
[a]HR per every 10-unit increase.

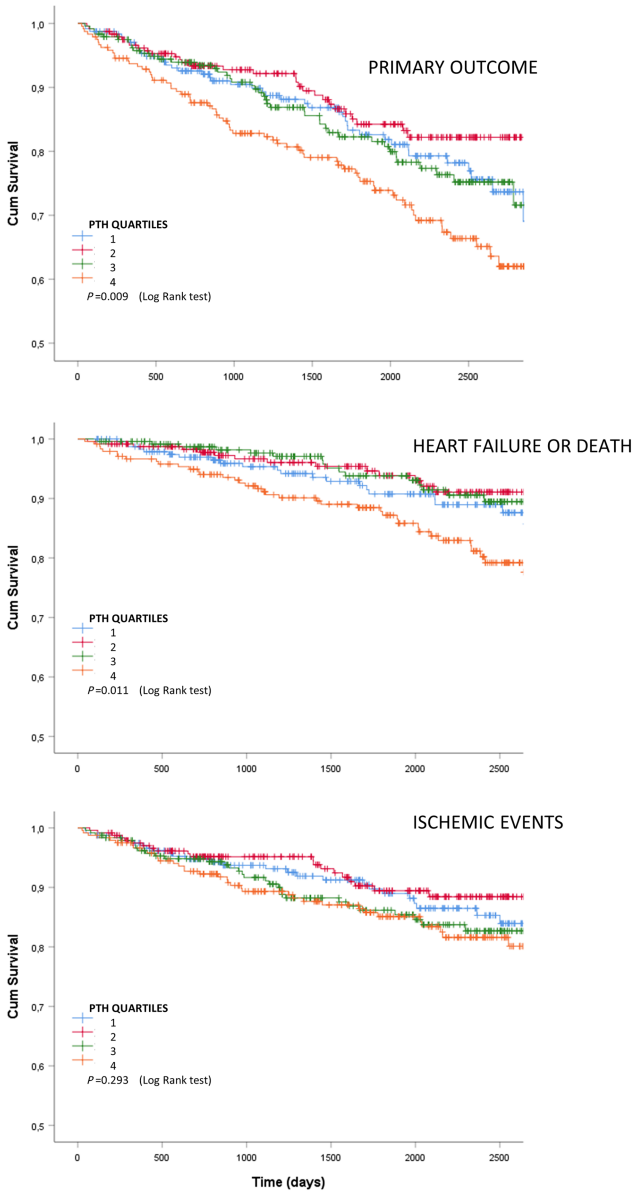
Correlation between components of mineral metabolism

We established the correlation between PTH and the other variables of the mineral metabolism, NT-proBNP and hs-TnI, in order to see if PTH could be predicted using the values of plasma levels of other components of mineral metabolism. However, all these correlations were poor (Supporting Information, *Figure S2*).

Discussion

Given its relationship with the kidney, mineral metabolism has traditionally been associated with CKD. However, abnormalities in mineral metabolism are common in coronary artery disease (CAD) patients, even in those with normal renal function, as we described in a previous cross-sectional study based in the first 704 patients of the BACS-BAMI study.[15,16] In addition, these abnormalities are associated with worse

Figure 1 Primary and secondary outcomes according to PTH quartiles. FGF23, fibroblast growth factor 23; PTH, parathormone.



cardiovascular prognosis.[2,4,16] This study was performed in 964 patients with stable CAD, of whom only 18% had CKD, and we demonstrate that abnormal levels of components of mineral metabolism are very common among these patients. However, our main objective was to explore the prognostic value of the different components of mineral metabolism, including soluble klotho, while controlling for other important cardiac markers such as NT-proBNP, hs-TnI, and hs-CRP.

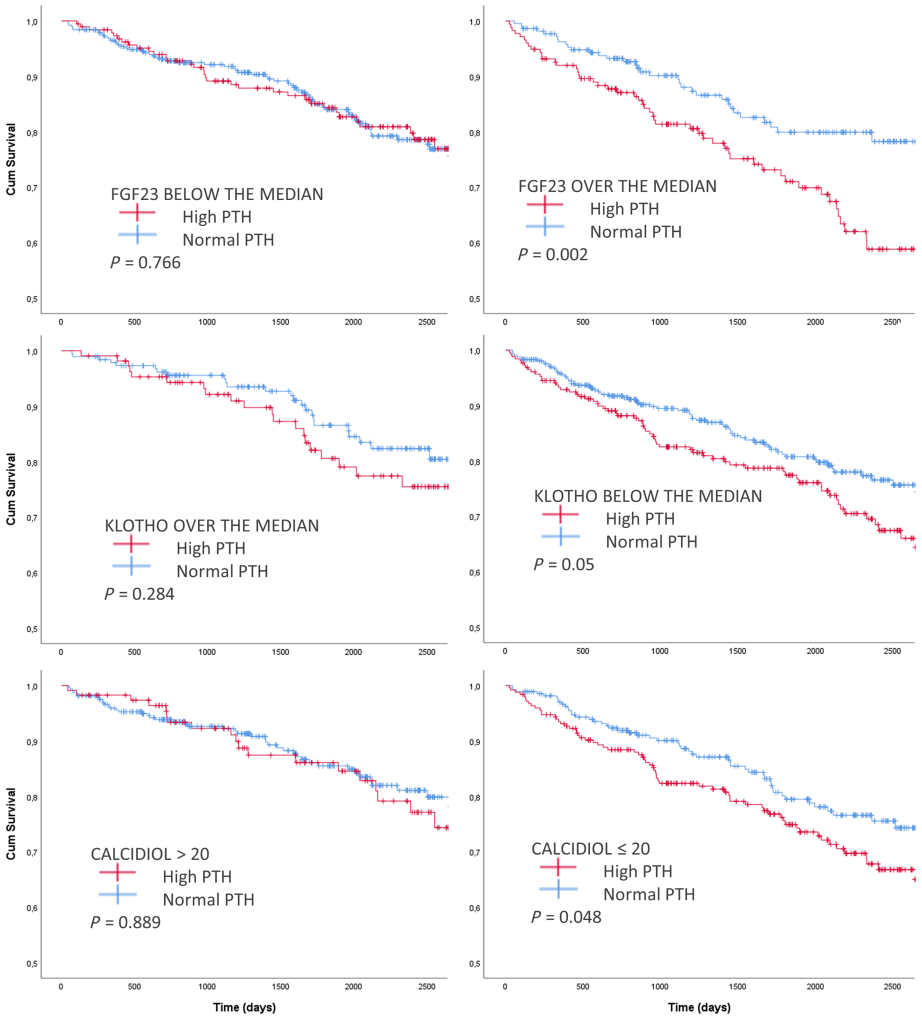
In this study, we have seen that PTH is an independent predictor of the primary endpoint of ischaemic events, heart failure, and death, and it also predicted the secondary outcomes of ischaemic events and the composite of heart failure and death, thereby adding value to other consistent markers. This is important, because NT-proBNP has been demonstrated to predict heart failure and death in different settings. Although these biomarkers are not used in stable CAD patients in clinical practice, we wanted to include them in our analysis in order to confirm that mineral metabolism markers add prognostic information even when the levels of these biomarkers are considered.

There is controversy surrounding the relationship between PTH and cardiovascular events. There are PTH receptors in myocytes which, when activated, may induce left ventricular hypertrophy.[8,17] This mechanism lends plausibility to the association observed in a variety of studies, including one a meta-analysis, between PTH levels and the development of left ventricular hypertrophy and heart failure,[6,18–20] while it contrasts with other recent studies which do not confirm such a relationship.[21,22] There is also a lack of consensus concerning the relationship between PTH and CAD. It has been shown that patients with primary hyperparathyroidism have coronary microvascular dysfunction, which is completely restored after parathyroidectomy,[23] and PTH levels have been linked to the incidence and progression of CAD,[24,25] and the incidence of cardiovascular events and mortality.[7,26,27] Conversely, other studies failed to confirm this association.[28,29]

We previously described with the first 704 patients of this cohort, and with shorter follow-up that an interaction between the predictive value of the components of mineral metabolism may exist, as low vitamin D levels predicted cardiovascular events in the presence of high FGF23 levels,[16] but not in cases with low FGF23. Furthermore, other groups have shown that this combination is also associated with a faster progression of CKD.[30] In the present study, we tried to confirm this hypothesis, and to do so, we divided the population into two groups according to FGF23 plasma levels. Of interest, PTH had a marked predictive value for the primary outcome exclusively in patients with high FGF23 levels, while it lost its predictive ability in patients with normal FGF23 levels and there was a significant interaction between PTH and FGF23 levels regarding the primary outcome. It is possible that some synergy between the effects of these two molecules on the heart exists. In this regard, the combination of high PTH and FGF-23 levels have been reported to be associated to an increased incidence of heart failure in the general population.[31] Also, FGF-23 levels are associated with decreased heart rate variability in CKD patients, but successful parathyroidectomy reverses this abnormality.[32] In addition, given that the correlation between PTH and FGF23 is poor, it is clear that we need to assess at least these two biomarkers in order to maximize prognostic ability.

Fibroblast growth factor-23 has been strongly related with cardiovascular diseases. It has been associated with the development of left ventricular hypertrophy and mortality in CKD patients[5,33] but also with heart failure in patients

Figure 2 Predictive power of PTH for the primary outcome according to the status of FGF23, soluble klotho, and calcidiol. FGF23, fibroblast growth factor 23; PTH, parathormone.



without significant CKD.[4] In the present study, FGF23 plasma levels were independently associated with the incidence of the primary outcome, but when hs-TnI and NT-proBNP were added to the model, it was no longer an independent predictor. However, rather than being a predictor of cardiovascular prognosis itself, it seems that it is related to the predictive power of PTH.

Vitamin D has been studied even more extensively than FGF23, revealing that it is inversely related to the extent of vascular calcification, severity of CAD, incidence of acute myocardial infarction, and cardiovascular death.[2,6,34,35] In addition, klotho is a co-receptor for FGF23 that may be found in soluble form in the plasma. It is thought to have protective cardio-renal effects, and its underexpression is associated with ectopic calcifications and aging.[10,36] Klotho produces a negative regulation of calcium channels, resulting in decreased phosphorus input to smooth muscle cells and less vascular calcification,[11] and klotho deficiency is associated with the development of left ventricular hypertrophy in rats.[5] Despite these effects, cohort studies failed to show any prognostic value for klotho.[37,38] In the present study, klotho and calcidiol plasma levels were inversely associated with the incidence of adverse outcomes but were not independent predictors of future adverse events. The extensive number of variables and biomarkers that we controlled for, including all components of mineral metabolism, may account for this result. Finally, the predictive value of PTH seemed to be present especially in patients with low klotho and calcidiol levels, while it disappeared in those with high plasma levels of these molecules. However, no significant interaction was found between PTH and both calcidiol and klotho plasma levels, suggesting that these do not share the ability of FGF23 to interact with the predictive power of PTH.

These findings should be considered when interpreting the results of clinical trials that failed to show a beneficial effect of treatment with vitamin D supplements.[39–41] One of the

reasons that could have determined these negative results may have been that an integrated analysis of other components of the mineral metabolism was not performed, because treatment with vitamin D supplements could have a beneficial effect only in those patients who also have disturbances in other components, mainly FGF23 and PTH. Therefore, it would be of interest to re-analyse trials in light of this issue to determine whether there are subgroups in which supplements of vitamin D are effective. Similarly, when designing future trials of vitamin D supplements, taking into account the baseline status of mineral metabolism may play a key role in the results obtained. Finally, monitoring the effect of vitamin D supplements on FGF23 and PTH should be performed in these trials, as a decrease in the levels of these biomarkers could indicate a beneficial clinical response.

The main limitation is that our study is based on a cohort of coronary patients with low percentage of previous heart failure and low ejection fraction. Thus, extrapolation of our results to populations with heart failure is not advisable. Five patients were lost during follow up, that is, 0.5% of all patients so we do not expect that affected the results of the study.

Conclusions

Parathormone is an independent predictor of cardiovascular events in patients with CAD, adding complimentary prognostic information to NT-proBNP plasma levels. This effect is limited to patients with high levels of FGF23, suggesting a relationship among the components of mineral metabolism regarding their prognostic value. Thus, this should be considered when designing and interpreting new studies in this area.

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Conflict of interest

José Tuñón has given lectures for Diasorin Spain.

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Supporting information

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

Table S1. Full baseline characteristics.
Table S2. Differences in the levels of the components of mineral metabolism according to eGFR.
Table S3. Univariate analysis.
Table S4. Interaction between PTH and other components of mineral metabolism in the prediction of the primary outcome.
Figure S1. Flow chart.
Figure S2. Correlations between PTH and other components of mineral metabolism, NT-proBNP and hs-TnI.

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