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Klotho plasma levels are an independent predictorof mortality in women with acute coronary syndrome

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Alterations in plasma levels of the components of the mineral metabolism (MM) system are related to cardiovascular diseases. However, gender differences of the whole MM system in patients with acute coronary syndrome (ACS) have not been reported. Our objective was to analyse the potential differences on the prognostic role of MM in women suffering an ACS as compared to men. We included 1,230 patients with ACS and collected clinical data and plasma levels of MM components. Primary outcome was a composite of acute ischaemic events, heart failure and all-cause mortality. Secondary outcomes included each component separately. 282 patients (22.9%) were female. After 5.44 years of follow-up, primary outcome occurred in 28.0% women and 23.5% men, and death in 10.6% and 9.4% respectively. FGF23 was associated with primary outcome in both sexes, and calcidiol only in men (HR 1.04, CI95%1.00-1.03). Klotho levels are inversely related to all-cause mortality only in women (HR 0.80, CI95% 0.67–0.96), while calcidiol (HR 0.84, CI95%0.72–0.98) and FGF23 levels (HR 1.02 CI95%1.00-1.03) were predictors in men, highlighting a possible gender-specific prognostic biomarker. These results underline the importance of considering MM biomarkers in risk stratification and management of patients with acute coronary syndromes, with attention to gender differences.

Keywords Klotho protein, Gender, Acute coronary syndrome, Mineral metabolism, Cardiovascular risk

The main role of the mineral metabolism (MM) system (calcidiol, fibroblast growth factor-23 [FGF23], phosphate, parathormone [PTH] and klotho) is to maintain mineral homeostasis. Specifically, FGF23 helps the failing kidneys to eliminate phosphorus and calcium[1]. It also promotes a reduction of the concentration of 1-25-dihydroxyvitamin D, that leads to a decrease in intestinal calcium absorption, stimulating PTH secretion. However, these compensatory changes of the different components of MM may promote the development of cardiovascular disorders (CVD), and their plasma levels may also have a prognostic role in certain CVD[2–5]. In the case of klotho, it works as a co-receptor of FGFR1 for the canonical actions of FGF23[6]. The non-canonical actions of FGF23, such as cytokine production and the development of cardiac hypertrophy and fibrosis, are mediated mainly by FGFR2-4 receptors without the participation of klotho. Then, a decrease of klotho levels

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favours an increase of the deleterious, non-canonical effects of FGF23[7], and it has been said that this molecule possesses protective effects[8–10].

In addition, these MM abnormalities are not limited to patients with chronic kidney disease (CKD), because they have been observed in patients with preserved renal function[11]. Furthermore, a prognostic role of abnormal plasma levels of MM has been demonstrated in subjects with average renal function[4,12–14].

Moreover, CVD is the most frequent cause of mortality worldwide. In this setting, there are important gender differences in acute coronary syndromes (ACS): different comorbidities, cardiovascular risk factors, differences in clinical presentation and in the quality of diagnostic and therapeutic medical management[15]. Even more, the risk of death is known to be higher in women, especially in the context of younger populations[16–18].

To our knowledge, there are no previous publications in the literature assessing the prognostic role of the whole MM after an ACS focusing on gender differences. In this work we have analysed the potential differences on the prognostic role of MM in women suffering an ACS as compared to men.

Methods

Patients and study design

We analysed the BACS & BAMI (Biomarkers in Acute Coronary Syndrome & Biomarkers in Acute Myocardial Infarction) study population, which included patients admitted to five hospitals in Madrid with ST-segment elevation myocardial infarction (STEMI) or non-ST-segment elevation acute coronary syndrome (NSTEACS, including non-STEMI and unstable angina). Inclusion and exclusion criteria have been detailed previously[19,20]. Between July 2006 and June 2014, a total of 2,740 patients were discharged from the study hospitals with a diagnosis of NSTEACS or STEMI. Of these, 1,483 patients were excluded based on the following predefined criteria: presence of survival-limiting toxic conditions or habits (29.8%), age > 85 years (16.4%),inability to complete follow-up (16.3%), clinical instability beyond day six after the index event (10.9%),inability of the investigators to include them (9.8%), inability to undergo cardiac revascularisation (9.6%), presence of other significant cardiac conditions (5.7%), and refusal to participate in the study (1.5%). Of the 1,257 patients included, 1,230 completed the follow-up (Fig. 1).

On admission, baseline clinical variables were documented, and 12-hour fasting venous blood samples were collected in EDTA tubes. These samples were centrifuged at 2500 g for 10 min, and the plasma was stored at -80 °C.

After discharge from hospital, all patients underwent annual assessments at their respective medical centres. At the conclusion of the follow-up period, medical records were reviewed, and patient status was confirmed through telephone contact. The last follow-up visits were conducted in June 2016.

The research protocol suited the ethical guidelines of the 1975 Declaration of Helsinki. This protocol was approved by the Ethics Committee of Fundación Jiménez Díaz University Hospital. The date of approval by this Ethics Committee was 24 April 2007 (act number 05-07). In addition, this protocol was also approved by the Ethics Committees of the other institutions participating in the study: Fundación Alcorcón Hospital, Fuenlabrada Hospital, Puerta de Hierro Majadahonda University Hospital, and Móstoles University Hospital. All participants were provided informed consent during the study.

Outcomes

The primary outcome was a composite of acute ischemic events (including non-STEMI, STEMI, unstable angina, ischaemic stroke, and transient ischemic attack), heart failure, and all-cause mortality. Secondary outcomes included each component of the primary outcome: acute ischemic events, heart failure, and death. NSTEACS was defined as rest angina lasting more than 20 min within the preceding 24 h, or new-onset class III-IV angina, accompanied by transient ST depression or T wave inversion on the electrocardiogram, as interpreted by the attending cardiologist, and/or elevated troponin levels. STEMI was defined by angina-like symptoms persisting for more than 20 min, ST elevation in at least two contiguous leads on the electrocardiogram, lack of response to nitroglycerin, and elevated troponin levels. A previous acute myocardial infarction was diagnosed in the presence of new pathological Q waves on the electrocardiogram, along with corresponding new myocardial scarring identified via echocardiography or nuclear magnetic resonance imaging. Heart failure was defined as the presence of typical symptoms, with or without signs, associated with left ventricular systolic dysfunction (< 50%) or, in case of preserved systolic function, associated with objective evidence of cardiac structural and/or functional abnormalities, including elevated natriuretic peptide levels. Ischaemic stroke was defined as the rapid onset of a neurological deficit attributable to a specific vascular territory, lasting more than 24 h, or confirmed by new ischemic lesions on imaging studies. A transient ischemic attack was characterized by transient neurological signs and symptoms of cerebral ischemia that resolved within 24 h, without acute ischemic lesions on imaging studies.

Although all events were recorded for each patient, only the first event was included in the Cox regression analysis. Therefore, while the total number of events is reported, patients who experienced multiple events were counted only once in these analyses.

Biochemical analysis

Plasma analyses were conducted at the Mineral Metabolism laboratory of La Paz Hospital and at the Vascular Pathology and Biochemistry laboratories of Fundación Jiménez Díaz University Hospital. The investigators responsible for these laboratory studies were blinded to the clinical data. Soluble-α-klotho levels (here in after referred to as "klotho") were assessed by ELISA (Human Soluble Alpha Klotho Assay Kit, Immuno-Biological Laboratories Co., Hokkaido, Japan). FGF23 was measured through an enzyme-linked immunosorbent assay (ELISA) that targets epitopes within the carboxyl-terminal region of FGF23 (Human FGF23, C-Term, Immutopics Inc, San Clemente, CA). Calcidiol plasma levels were quantified using a chemiluminescent immunoassay on the
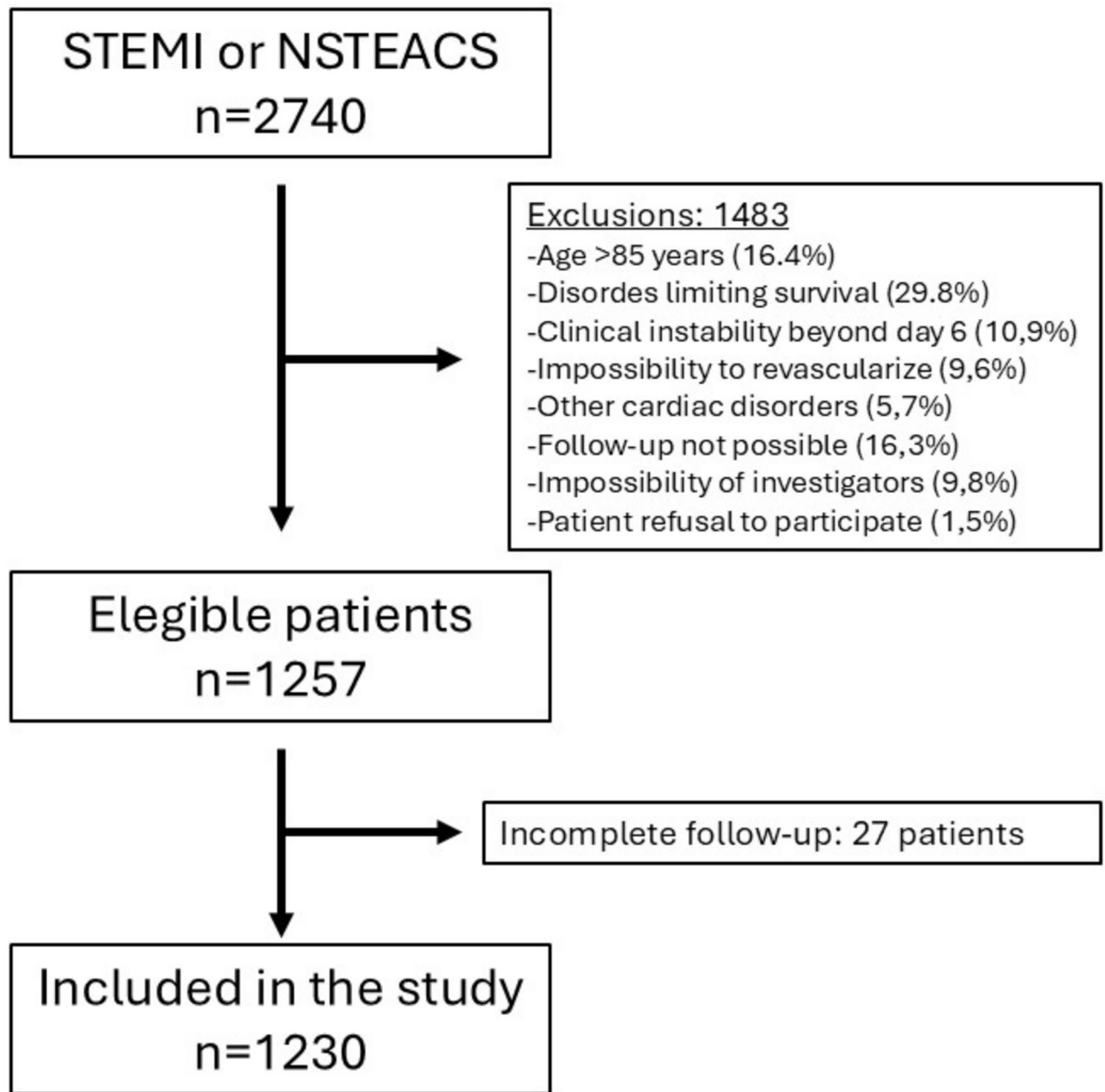
Fig. 1. Study flow chart.

LIAISON XL analyzer (LIAISON 25OH-Vitamin D Total Assay, DiaSorin, Saluggia, Italy). Intact parathyroid hormone (PTH) was analyzed using a second-generation automated chemiluminescent method (Elecsys 2010 platform, Roche Diagnostics, Mannheim, Germany), and phosphate levels were determined by an enzymatic method (Integra 400 analyzer, Roche Diagnostics, Mannheim, Germany). N-terminal pro-brain natriuretic peptide (NT-proBNP) levels were measured via immunoassay (VITROS, Ortho Clinical Diagnostics, Raritan, NJ, USA), troponin I through an immunometric immunoassay using a biotinylated monoclonal mouse antibody and a luminescent reaction (Ortho Clinical Diagnostics Vitros XT 7600, Raritan, NJ, USA), and high-sensitivity C-reactive protein (hs-CRP) by latex-enhanced immunoturbidimetry (ADVIA 2400 Chemistry System, Siemens, Munich, Germany). Lipid, glucose, and creatinine levels were determined using standard methods (ADVIA 2400 Chemistry System, Siemens, Munich, 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, and those with a not normal distribution are displayed as median (interquartile range). Categorical variables are expressed using frequency measurements (absolute frequencies and percentages).

A baseline comparative analysis of variables was conducted based on gender. Categorical data were evaluated using the χ^2 test or Fisher's exact test. For continuous variables, a Student's t-test was applied to those with a normal distribution, while the Mann–Whitney U test was employed for those not normally distributed. A p-value of less than 0.05 was considered indicative of statistical significance.

A univariate Cox regression analysis was conducted to determine which variables were associated with the development of various outcomes, separately for men and women. Subsequently, a multivariate regression analysis was performed to identify significant predictors of clinical outcomes in both genders. The selection criteria for variables included in the multivariate analysis were based on clinical and biological plausibility, as well as statistical significance observed in the univariate analyses. The magnitude of the effects of the variables was expressed in the form of hazard ratios (HRs) and 95% confidence intervals (CIs).

All analyses were conducted using the Statistical Package for the Social Sciences (SPSS v.26.0, IBM, Armonk, NY, USA), the R statistical language version 4.0.5 (R Foundation for Statistical Computing, Vienna, Austria) and the statistical package for the biomedical sciences (MedCalc v.23.0.2, Ostend, Belgium; https://www.medcalc.org).

Results
Baseline characteristics
1,230 patients were included in our study. Of these, 282 (22.9%) were women. Women were older than men (65.7 vs. 60.5 years, $p < 0.001$), with a higher percentage of hypertension (70.6% vs. 53.2%), and of CKD prior to admission (28.7% vs. 15.9%) (Table 1). On the other hand, men had a significantly higher rate of smokers (46.6% vs. 28.0%), with more coronary heart disease and peripheral arterial disease prior to inclusion in the study. In both genders, the percentage of STEMI included in our population was around 50%. On average, men had a greater number of affected vessels (1.51 vs. 1.22, $p < 0.001$), and a higher percentage of them received revascularization treatment. This translated into a lower proportion of women with P2Y12 inhibitors at discharge (85.5% vs. 91.6%, p 0.004), with no other difference in treatment at discharge between men and women. Regarding the biomarkers analyzed, women generally presented significantly higher levels of PTH, FGF23, klotho, phosphorus, and NT-proBNP. Median time for blood extraction from admission was 4 (2–5) days.

Primary outcome
Median follow-up was 5.44 (3.03–7.46) years. During follow-up, 79 women (28.0%) and 223 men (23.5%) developed a primary event (a composite of acute ischemic events, heart failure, and all-cause mortality).

We performed a multivariate Cox regression analysis of our study population to identify independent predictors of primary outcomes separately for men and women, as described previously. This analysis revealed that FGF23 levels were directly and significantly related to the occurrence of a primary outcome in both sexes (Table 2). PTH also showed a relationship with primary outcome in the male group, but not in the female group. Other variables (clinical or treatment) also showed a significant relationship with outcomes in both groups, although no other biomarker showed a significant relationship.

Secondary outcomes
At the end of follow-up, 55 women (19.5%) and 133 men (14%) presented an acute ischemic event. For each of these groups, we performed a multivariate Cox regression analysis to identify significant predictors of this outcome (Table 3). Variables such as eGFR, heart failure (prior to inclusion), hypertension, etc. were shown to be a predictor of acute ischemic events. However, none of the analyzed biomarkers showed a statistically significant relationship in either group.

Twenty women (7.1%) and 48 men (5.1%) developed heart failure during follow-up. After multivariate Cox regression analysis, again FGF23 was an independent risk factor for the development of heart failure in both men and women (Table 4). PTH also showed a statistically significant relationship with this event but as observed for the primary outcome, only in the male population.

Finally, we analyzed the variables related to all-cause mortality in both genders. By the end of follow-up, 30 women (10.6%) and 89 men (9.4%) had died. We observed that klotho was an independent protective factor in the female population for all-cause mortality (HR 0.80, CI95% 0.67–0.96; $p = 0.019$) (Table 5). Phosphate levels were also independently but positively associated with this outcome in this population (HR 2.24 (1.11–4.50; $p = 0.025$). In contrast, in men the MM components associated with this outcome included only FGF23 (HR 1.02 (1.00-1.03); $p = 0.048$) and calcidiol (HR 0.84 (0.72–0.98); $p = 0.024$) but not klotho and phosphate plasma levels.

Discussion
Many differences have been described between men and women regarding coronary artery disease. Women have less coronary atherosclerosis[21], and a lower risk of suffering ACS than men[22]. In addition, the age of onset is higher in women than in men, with a higher prevalence of comorbidities[23]. The impact of the different risk factors according to gender also presents differences; with the greater importance in women of diabetes, hypertension and smoking[24–26]. Thus, the risk of death is known to be higher in women. This higher mortality in women is described mainly in the earlier phases after ACS, with a worse prognosis and higher mortality having been observed immediately after percutaneous intervention[23,27]. The possible causes of this worse prognosis are diverse, and they may include a delay in the diagnosis of ACS favored by the higher likelihood of atypical symptoms[28] and differences in high-sensitivity troponin levels as compared to men[29]. Furthermore, women with ACS are less likely to receive optimal therapy than men[30–34].

Changes in plasma levels of the different components of MM have been related to different cardiovascular abnormalities. Low calcidiol and high PTH levels have been associated with left ventricular hypertrophy,

Variable	Female ($n = 282$)	Male ($n = 948$)	p
Age (years)	65.7 ± 13.0	60.5 ± 11.6	< 0.001
Age > 70 years	127 (45.0%)	241 (25.4%)	< 0.001
Caucasian race	275 (97.5%)	917 (96.7%)	NS
Diabetes	70 (24.8%)	210 (22.2%)	NS
Hypertension	199 (70.6%)	504 (53.2%)	< 0.001
Body mass index (kg/m^2)	28.5 ± 5.16	28.3 ± 4.02	NS
Dyslipidaemia	165 (58.5%)	575 (60.7%)	NS
Smoking	79 (28.0%)	442 (46.6%)	< 0.001
eGFR < 60 mL/min/1.73 m^2	81 (28.7)	151 (15.9)	< 0.001
Heart failure (prior to inclusion)	4 (1.4%)	10 (1.1%)	NS
Coronary artery disease (prior)	42 (14.9%)	202 (21.3%)	0.022
Peripheral artery disease (prior)	7 (2.5%)	64 (6.8%)	0.011
Cerebrovascular accident (prior)	13 (4.6%)	27 (2.8%)	NS
Atrial fibrillation (prior)	8 (2.8%)	20 (2.1%)	NS
Laboratory results			
Hemoglobin (g/dl)	13.5 ± 1.50	15.1 ± 1.88	< 0.001
Glycaemia (mg/dL)	116 ± 38.4	117 ± 39.1	NS
eGFR (mL/min/1.73 m^2)	74.9 ± 22.3	79.8 ± 19.2	0.001
Troponin (ng/mL)	8.13 (0.80, 30.1)	11.4 (0.82, 59.4)	0.022
LDL (mg/dL)	119 ± 39.4	116 ± 36.1	NS
Triglycerides (mg/dL)	122 (82.5, 177)	134 (98.0, 185)	0.006
HDL (mg/dL)	47.2 ± 14.2	38.1 ± 10.2	< 0.001
Calcidiol (ng/mL)	16.4 (12.4, 24.6)	18.6 (13.5, 24.7)	0.095
PTH (pg/mL)	54.0 (40.3, 70.0)	45.0 (36.0, 60.0)	< 0.001
FGF23 (RU/mL)	126 (95.0, 173)	105 (82.0, 138)	< 0.001
Klotho (pg/mL)	657 ± 219	621 ± 205	0.013
Phosphorus (mg/dL)	3.44 (3.12, 3.82)	3.21 (2.85, 3.58)	< 0.001
NT-proBNP (pg/mL)	551 (226, 1500)	348 (121, 912)	< 0.001
hs-CRP (ng/dL)	1.36 (0.59, 2.85)	1.74 (0.78, 3.62)	0.012
Type of ACS and coronary findings			
NSTEMI	114 (40.4%)	326 (34.4%)	NS
STEMI	129 (45.7%)	482 (50.8%)	NS
Unstable angina	39 (13.8%)	140 (14.8%)	NS
LVEF < 40%	41 (14.6%)	131 (13.8%)	NS
Number of affected vessels	1.22 ± 0.80	1.51 ± 0.79	< 0.001
Left main disease	9 (3.3%)	33 (3.5%)	NS
Complete revascularization	211 (74.8%)	660 (69.6%)	NS
Type of revascularization			< 0.001
No revascularization	70 (24.8%)	115 (12.1%)	
Drug eluting stent	142 (50.4%)	492 (51.9%)	
Bare metal stent	52 (18.4%)	261 (27.5%)	
Balloon angioplasty	11 (3.9%)	27 (2.8%)	
Coronary artery bypass grafting	7 (2.5%)	53 (5.6%)	
Treatments at discharge			
ASA	264 (93.6%)	911 (96.2%)	NS
P2Y12 inhibitors	241 (85.5%)	867 (91.6%)	0.004
Anticoagulant	22 (7.8%)	60 (6.3%)	NS
Statins	264 (93.6%)	916 (96.7%)	NS
Ezetimibe	4 (1.4%)	18 (1.9%)	NS
Insulin	25 (8.9%)	57 (6.0%)	NS
Oral antidiabetic drugs	39 (13.8%)	149 (15.7%)	NS
Continued			

Variable	Female($n = 282$)	Male ($n = 948$)	p
ACEI/ARB	227 (80.5%)	770 (81.3%)	NS
MRAs	26 (9.2%)	71 (7.5%)	NS
Beta-blockers	227 (80.5%)	789 (83.4%)	NS
Nitrates	53 (18.8%)	133 (14.0%)	NS
Diuretics	48 (17.0%)	139 (14.7%)	NS

Table 1. Baseline characteristis of study population. ACEI: angiotensin converting enzyme inhibitor; 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; NS: statistically not significant; NSTEMI: non-ST elevation myocardial infarction; MRAs: mineralocorticoid receptor antagonists; NT-ProBNP: N-terminal-probrainnatriuretic peptide; PTH: parathormone; STEMI: ST-elevation myocardial infarction.

Gender	Variable	HR (CI 95%)	p
Female	eGFR	0.85 (0.77–0.95)	0.006
	Nitrates	2.34 (1.40–3.90)	0.001
	CVA	4.17 (1.98–8.78)	< 0.001
	FGF23	1.02 (1.01–1.04)	0.001
	Diltiazem	2.41 (1.07–5.40)	0.034
	Diabetes	1.65 (1.02–2.66)	0.040
	Statins	0.497 (0.25–0.99)	0.049
Male	Age	1.0 (1.02–1.04)	< 0.001
	Diuretics	1.77 (1.29–2.43)	< 0.001
	CAD	1.40 (1.05–1.88)	0.023
	Diabetes	1.50 (1.13–2.01)	0.006
	ASA	0.52 (0.31–0.86)	0.012
	PTH	1.06 (1.01–1.11)	0.016
	FGF23	1.04 (1.00-1.03)	0.027

Table 2. Multivariable Cox regression analysis for the primary outcome of acute ischemic event, heart failure or death. ASA: acetylsalicylic acid; CVA: cerebrovascular accident (prior to inclusion); CAD: coronary artery disease (prior to inclusion); eGFR: estimated glomerular filtration rate; FGF23: fibroblast growth factor 23; HR: Hazar Ratio; PTH: parathormone.

Gender	Variable	HR (CI 95%)	p
Female	Heart failure	6.43 (2.09–19.79)	0.002
	Nitrates	3.19 (1.79–5.66)	< 0.001
	Glycaemia	1.09 (1.03–1.15)	0.004
	eGFR	0.87 (0.76–0.98)	0.028
Male	CCB	2.43 (1.59–3.40)	< 0.001
	Heart failure	3.75 (1.37–10.29)	0.011
	Hypertension	1.75 (1.20–2.57)	0.004
	Triglycerides	1.01 (1.00-1.02)	0.018
	ACEI/ARB	0.65 (0.44–0.97)	0.033

Table 3. Multivariable Cox regression analysis for the secondary outcome of acute ischemic events. ACEI: angiotensin converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker; eGFR: estimated glomerular filtration rate; HR: Hazar Ratio.

hypertension, coronary heart disease and increased cardiovascular risk[2,20,35]. Increased FGF23 has been related with left ventricular hypertrophy[3,36,37], heart failure[38,39], atrial fibrillation[40] and coronary heart disease[4] and is associated with worse prognoses both in the general population and in patients with CVD[5,41–44]. Furthermore, MM abnormalities are not restricted to patients with CKD, but they are also present in patients with normal renal function[11], where they may also have prognostic value[4,12–14]. In spite of this, there is no information regarding the behaviour of MM components in women with ACS.

Gender	Variable	HR (CI 95%)	p
Female	MRAs	9.22 (3.21–26.55)	<0.001
	FGF23	1.03 (1.01–1.06)	0.009
	Insulin	5.56 (1.71–18.31)	0.008
	ASA	0.23 (0.06–0.94)	0.042
Male	FGF 23	1.04 (1.03–1.06)	<0.001
	PTH	1.14 (1.06–1.23)	0.002
	Age	1.06 (1.02–1.09)	0.001
	Oral antidiabetic drugs	3.02 (1.60–5.71)	0.001
	LVEF < 40%	3.73 (1.99–6.95)	<0.001
	Diltiazem	4.97 (1.68–14.69)	0.005
	Hypertension	2.23 (1.03–4.84)	0.043

Table 4. Multivariable Cox regression analysis for the secondary outcome of heart failure. ASA: acetylsalicylic acid; eGFR: estimated glomerular filtration rate; FGF23: fibroblast growth factor 23; LVEF: left ventricular ejection fraction; MRAs: mineralocorticoid receptor antagonists; HR: Hazar Ratio; PTH: parathormone.

Gender	Variable	HR (CI 95%)	p
Female	eGFR	0.97 (0.95–0.98)	0.001
	Anticoagulant	6.77 (2.69–17.05)	<0.001
	Diuretics	3.67 (1.58–8.51)	0.004
	P2Y12 inhibitors	0.33 (0.14–0.77)	0.013
	Phosporus	2.24 (1.11–4.50)	0.025
	Klotho	0.80 (0.67–0.96)	0.019
	LVEF < 40%	2.45 (1.05–5.76)	0.040
Male	Age	1.10 (1.07–1.13)	<0.001
	Insulin	2.59 (1.44–4.66)	0.002
	NT-proBNP	1.02 (1.01–1.03)	0.002
	Heart failure (prior)	4.28 (1.78–10.27)	0.001
	ASA	0.41 (0.20–0.84)	0.015
	Smoking	1.71 (1.00-2.92)	0.049
	Calcidiol	0.84 (0.72–0.98)	0.024
	FGF23	1.02 (1.00-1.03)	0.048

Table 5. Multivariable Cox regression analysis for the secondary outcome of all-cause mortality. ASA: acetylsalicylic acid; eGFR: estimated glomerular filtration rate; FGF23: fibroblast growth factor 23; LVEF: left ventricular ejection fraction; HR: Hazar Ratio; NT-ProBNP: N-terminal-probrain natriuretic peptide.

In this paper we demonstrate that women with ACS present a worse MM profile than men, with higher FGF23, PTH, and phosphate levels. Regarding prognosis, FGF23 levels were independent predictors of the primary outcome in both genders, while PTH added independent predictive value only in men. Similar results were obtained in the prediction of heart failure, where PTH showed significant predictive value in the multivariate study only in males (in agreement with the observations of other authors where PTH level was related to clinical and subclinical markers of congestion[45], but not in females. However, for the prediction of all-cause mortality there were marked differences between both groups, with low klotho levels being predictive in women, along with phosphate levels, while in men high FGF23 and low calcidiol levels were the independent predictors for this outcome.

Klotho is the co-receptor of FGFR1 for FGF23, which exerts its beneficial actions through this canonical pathway, such as helping the failing kidneys to eliminate phosphate. However, in the absence of klotho, FGF23 binds to other receptors promoting cardiac hypertrophy and fibrosis and stimulating the production of pro-inflammatory cytokines[7]. Then, it has been said to have protective effects. Klotho suppression in animal models is associated with early ageing, shorter life expectancy, multi-organ dysfunction and marked alterations in mineral homeostasis (hyperphosphataemia, hypercalcaemia, among others.)[46,47]. More recently, an independent association between low klotho levels with left ventricular hypertrophy[48], heart failure[49–52], atrial fibrillation[53] and myocardial infarction[50] has been described in humans. Klotho has also demonstrated a prognostic role in heart failure[49,54], with low levels also being a marker of total and cardiovascular mortality in CKD patients[55] and in the general population[56,57].

Basic research studies report results that may explain in part a potential benefit of klotho in ACS. The administration of klotho in animal models protects the myocardium from ischaemic and reperfusion damage

through the activation of different pathways that reduce oxidative stress and restore autophagy levels[58,59]. Klotho has been described to modulate platelet activity[60]. Klotho therapy improves cardiac remodelling in a murine model of myocardial infarction[61] and it also ameliorates diastolic function[62], although there are not clinical studies confirming these findings. In several other studies, authors have also observed a beneficial effect of klotho on the vascular endothelium, reducing oxidative stress at this level or attenuating cell apoptosis, among other mechanisms[63–66].

However, there are no published data on the prognostic role of klotho after an ACS. To our knowledge, our work is the first to describe an independent protective role of klotho after ACS. According to the present findings, we have recently shown that cardiac rehabilitation after ACS is associated with increases of klotho levels[67], suggesting an increase in klotho could explain, at least in part, the benefits of cardiac rehabilitation. Of interest, the protective role of klotho is limited to women. It is possible that the higher cardiovascular risk profile of men, with more extensive coronary disease and a greater number of affected vessels, may interfere with the prognostic role of klotho, attenuating its protective effect after ACS. It is also striking that our results show that other MM biomarkers have a prognostic value in men but not in women. Nevertheless, we have demonstrated previously that it is common that several MM biomarkers have independent prognostic value[14,41], even after adjusting for established biomarkers such as NT-proBNP. This underlines the need to make a complete assessment of MM to investigate the prognostic value of its components.

Limitations

First, the percentage of women in our study population is relatively low. This is in line with many other published studies. There are several factors that may influence the lower recruitment of women, among them, a higher prevalence of ACS in men could partly account for this difference. Second, 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 led to the exclusion 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. Third, the inclusion period ended in 2014. Since then, new pharmacological and non-pharmacological treatments (stents, catheters, etc.) have emerged that could influence the results of similar studies in current populations. Fourth, almost all the women included in our study population were in the postmenopausal period. However, the variables collected in our study did not include the presence of osteoporosis or the use of specific gynaecological therapies. It is possible that hormonal or osteoporosis treatments may influence some of the parameters and/or biomarkers obtained.

Conclusions

In conclusion, our results show that klotho levels are inversely related to all-cause mortality in women after an ACS, highlighting a possible gender-specific prognostic biomarker. These results underline the importance of considering MM biomarkers in the risk stratification and management of ACS patients, with attention to gender differences. Future research should explore the underlying mechanisms of these associations.

Data availability

Data is provided within the manuscript. Further information and requests for resources and data base should be directed to and will be fulfilled by the lead contact, Dr.Marcelino Cortés (mcortesg@quironsalud.es).

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The authors declare no competing interests.

Additional information

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