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Original article

Differential profile in inflammatory and mineral metabolism biomarkers in patients with ischemic heart disease without classical coronary risk factors



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ABSTRACT

Background: Patients with coronary heart disease (CHD) without classical cardiovascular risk factors (CRFs) are uncommon, and their profile has not been thoroughly studied. In CHD patients, we have assessed the differences in several biomarkers between those with and without CRF.

Methods: We studied 704 patients with CHD, analyzing plasma levels of biomarkers related to inflammation, thrombosis, renal damage, and heart failure: high-sensitivity C-reactive protein (hs-CRP), monocyte chemoattractant protein-1 (MCP-1), galectin-3, N-terminal fragment of brain natriuretic peptide (NT-pro-BNP), calcidiol (vitamin D metabolite), fibroblast growth factor-23 (FGF-23), parathormone, and phosphate. **Results:** Twenty patients (2.8%) exhibited no CRFs. Clinical variables were well balanced in both groups, with the logical exceptions of no use of antidiabetic drugs, lower triglyceride and glucose, and higher high-density lipoprotein cholesterol in no-CRF patients.

No-CRF patients showed lower hs-CRP (2.574 ± 3.120 vs. 4.554 ± 9.786 mg/L; $p = 0.018$), MCP-1 (114.75 ± 36.29 vs. 143.56 ± 65.37 pg/ml; $p = 0.003$), and FGF-23 (79.28 ± 40.22 vs. 105.17 ± 156.61 RU/ml; $p = 0.024$), and higher calcidiol (23.66 ± 9.12 vs. 19.49 ± 8.18 ng/ml; $p = 0.025$) levels. At follow-up, 10.0% vs. 11.0% patients experienced acute ischemic event, heart failure, or death in the non-CRF and CRF groups, respectively ($p = 0.815$, log-rank test). The limited number of non-CRF patients may have influenced this finding. A Cox regression analysis in the whole population showed that high calcidiol, and low MCP-1 and FGF-23 plasma levels are associated with a better prognosis.

Conclusions: CHD patients without CRFs show a favorable biomarker profile in terms of inflammation and mineral metabolism. Further studies are needed to investigate whether this difference translates into a better prognosis.

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Introduction

The importance of cardiovascular risk factors (CRFs) in the development of coronary heart disease (CHD) is well established, and it is widely recognized that they must be controlled in order to halt the progression of the disorder [1]. However, some patients with CHD do not present with classical CRFs. Although these patients are managed similarly to patients who have CRFs, they sometimes show differences as compared to those with CRFs. Despite this, less attention has been paid to this subgroup.

In recent years there has been a growing interest in the role of prognostic biomarkers in atherothrombosis. It has been shown that increased levels of high-sensitivity C-reactive protein (hs-CRP), monocyte chemoattractant protein-1 (MCP-1), galectin-3, and N-terminal probrain natriuretic peptide (NT-proBNP) are related to an adverse prognosis [2–8]. More recently, plasma levels of the components of mineral metabolism, such as vitamin D, fibroblast growth factor-23 (FGF-23), parathormone, and phosphate have also been related to cardiovascular disorders [9–12].

We have investigated whether CHD patients who do not have CRFs present a more favorable profile of prognostic biomarkers related to atherothrombosis. We divided a population of 704 patients with CHD into those with and without CRFs. These patients were examined according to a panel of biomarkers related to inflammation, thrombosis, renal damage, and heart failure: hs-CRP, MCP-1, galectin-3, and NT-pro-BNP [2–8]. In addition, we determined the levels of the components of mineral metabolism in the patients' plasma: calcidiol (a metabolite of vitamin D), FGF-23, parathormone, and phosphate.

Methods

Patients

The research protocol was approved by the ethics committees of the participating hospitals and all patients signed informed consent documents. The BACS & BAMl (Biomarkers in Acute Coronary Syndrome & Biomarkers in Acute Myocardial Infarction) studies included patients admitted to four hospitals in Madrid who had either non-ST elevation acute coronary syndrome or ST-elevation acute myocardial infarction, as described previously [5]. Exclusion criteria were: age over 85 years, coexistence of other significant cardiac disorders except left ventricular hypertrophy secondary to hypertension, coexistence of any illness or toxic habits that could limit patient survival, impossibility to perform revascularization when indicated, and subjects in whom follow-up was not possible. In order to limit the variability of the findings due to an excessive heterogeneity in the intervals between the acute event and blood extraction, the investigators agreed to exclude patients who were not clinically stable at day six of the index event.

In addition to plasma withdrawal at discharge, a second plasma sample was extracted between 6 and 12 months later, on an outpatient basis. This paper reports data from the clinical and analytic findings obtained during this second plasma extraction.

Between July 2006 and April 2010, 1898 patients who had experienced an acute coronary event were discharged from the participating hospitals. Of these, 838 were included in the study. The remaining patients were not included due to one of the following reasons: age over 85 years (17.3%), disorders or toxic habits limiting survival (29.0%), impossibility to perform cardiac revascularization (14.5%), coexistence of other significant cardiopathy (6.8%), impossibility to perform follow-up (12.0%), clinical instability beyond the sixth day at the index event (9.1%), refusal to participate in the study (2.0%), and impossibility of the investigators to include them (9.3%). Of the patients included, 7 died before the second

plasma extraction and 709 had available and adequate plasma samples at the time. This visit took place between January 2007 and February 2011. The patients were included in a follow-up study, with the last visits taking place in May 2012. Five patients were lost to follow-up, leaving a total of 704 patients for analysis.

Study design

The cross-sectional study was carried out during the visit performed 6–12 months after the acute coronary event. Blood was withdrawn from the patients for plasma storage and a complete set of clinical variables was recorded. Twelve-hour fasting venous blood samples were collected in ethylenediaminetetraacetate tube (EDTA). Patients were considered to present with hypertension if they had a history of systolic and/or diastolic pressure equal to or higher than 140 and 90 mmHg, respectively [13] or if they were taking blood-pressure-lowering drugs for this disorder. Patients with current or past tobacco use were considered smokers. Patients receiving lipid-lowering therapy for this diagnosis and those with fasting lipid levels (low-density lipoprotein cholesterol >160 mg/dl and/or triglyceride levels >200 mg/dl) were considered to be diagnosed with dyslipidemia [14]. Patients were considered to present a family history of atherosclerosis when a first-degree relative younger than 60 years had history of CHD, peripheral vascular disease, or cerebrovascular disease [15]. Finally, patients were considered to be diabetics if they were receiving therapy for the condition or if they had fasting glucose levels >126 mg/dl [16].

A prospective observational study was also initiated during this visit. Under the study, patients were seen every year at their hospital. At the end of follow-up (maximum 4.6 years), the patients' medical records were reviewed and patient status was confirmed by telephone contact made by a cardiologist. The outcome variable was the combination of acute thrombotic events (any acute coronary syndrome, stroke, and transient ischemic attack) plus all-cause mortality and heart failure, defined as described previously [5].

Biomarker and analytical studies

Venous blood samples were centrifuged at 2500 g for 10 min. Plasma was stored at -80°C in the biobank of IIS-Fundación Jiménez Díaz. Plasma determinations of calcidiol, FGF-23, and intact parathormone were performed at the laboratory of Nephrology and Mineral Metabolism at the Gómez-Ulla hospital. The remaining determinations were carried out at the Vascular Pathology and Biochemistry Laboratories 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) using the LIAISON[®] XL analyzer (LIAISON 25OH Vitamin D total Assay, DiaSorin, Saluggia, Italy), FGF-23 was measured by an enzyme-linked immunosorbent assay that recognizes epitopes within the carboxyl-terminal portion of FGF-23 (Human FGF-23, C-Term, Immotopics Inc., San Clemente, CA, USA), intact parathormone was analyzed by a second-generation automated chemiluminescent method (Elecys 2010 platform, Roche Diagnostics, Mannheim, Germany), and phosphate was determined through an enzymatic method (Integra 400 analyzer, Roche Diagnostics). Plasma concentrations of MCP-1 and galectin-3 were determined in duplicate using commercially available enzyme-linked immunosorbent assay kits (BMS279/2 Bender MedSystems, Burlingame, CA, USA, and DCP00, R&D Systems, Minneapolis, MN, USA, respectively) following the manufacturers' instructions. Intra- and inter-assay coefficients of variation were 4.6% and 5.9% for MCP-1 and 6.2% and 8.3% for galectin-3, respectively. Hs-CRP was assessed by latex-enhanced

immunoturbidimetry (ADVIA 2400 Chemistry System, Siemens, Erlangen, Germany), and NT-pro-BNP was analyzed by immunoassay (VITROS, Orthoclinical Diagnostics, Raritan, NJ, USA). Lipids, glucose, and creatinine determinations were performed by standard methods (ADVIA 2400 Chemistry System).

Statistical analysis

Quantitative data that followed a normal distribution are presented as mean \pm standard deviation and compared using the Student “t” test. When normal distribution could not be assumed, median (interquartile range) and Mann–Whitney test were used. Qualitative variables are displayed as percentages and were compared by χ^2 or Fisher’s exact test where appropriate. Log-rank test was used to compare time to outcome in patients without CRFs against those who had CRFs. Cox regression analysis was performed with forward, stepwise selection, to assess the predictive value of the

biomarkers studied for the development of the outcome, controlling for all the variables shown in Table 1. Analyses were performed with SPSS 19.0 (SPSS Inc., New York, NY, USA) and R 3.0.1. (<http://www.r.project.org/>).

Results

Of the 704 patients included, only 20 (2.8%) did not present any classical CRFs. Forty percent of these patients were women (Table 1). Age was similar in the non-CRF and the CRF groups, and there were no significant differences in the clinical variables between both groups, with the exception of the intake of antidiabetic drugs, which was more frequent in the CRF group (18.3% vs. 0%; $p = 0.034$). Glucose (93.7 mg/dl vs. 109.6 mg/dl; $p = 0.001$) and triglycerides (95.5 ± 40.3 mg/dl vs. 132.1 ± 83.6 mg/dl; $p < 0.001$) plasma levels were lower in the non-CRF group than in patients with CRFs. On the other hand, high-density lipoprotein

Table 1
Characteristics of patients with and without cardiovascular risk factors.

Characteristics	Patients without CRF (N=20)	Patients with CRF (N=684)	p-Value	Patients with CRF (N=40) ^a	p-Value ^b
Age (years)	63.45 \pm 11.92	61.35 \pm 12.28	0.450	64.58 \pm 7.75	0.704
Male sex (%)	60.0	75.7	0.118	60.0	1.000
Family history atherosclerosis (%)	20	24.7	0.795	32.5	0.375
Body Mass Index	28.13 \pm 4.09	28.73 \pm 4.54	0.557	29.07 \pm 5.6	0.511
Cerebrovascular events (%)	0.0	3.5	1.000	2.5	1.000
Previous PAD (%)	0.0	3.9	1.000	2.5	1.000
Previous CABG (%)	5.0	8.5	1.000	12.5	0.653
Atrial fibrillation	0.0	5.0	0.617	0.0	–
Ejection fraction <40% (%)	15.0	11.7	0.721	12.5	1.000
ASA (%)	100.0	91.7	0.396	90.0	0.291
Clopidogrel (%)	75.0	67.5	0.630	70.0	0.769
Acenocoumarol (%)	0.0	6.0	0.623	2.5	1.000
Statins (%)	95.0	87.4	0.495	87.5	0.653
Oral antidiabetic drugs (%)	0.0	18.3	0.034	10.0	0.291
Insulin (%)	0.0	6.6	0.632	2.5	1.000
ACEI/ARB (%)	60.0	70.6	0.325	72.5	0.384
Aldosterone receptor blockers (%)	10.0	5.7	0.327	0.0	0.107
Beta-blockers (%)	70.0	76.6	0.592	77.5	0.542
Verapamil (%)	0.0	0.4	1.000	0.0	–
Diltiazem (%)	0.0	3.4	1.000	5.0	0.548
Dihydropyridines (%)	10.0	16.8	0.554	22.5	0.307
Data at the previous acute coronary event					
Complete revascularization (%)	70.0	65.2	0.770	65.0	0.750
Number of vessels diseased ^c			0.439		0.230
0 (%)	10.0	9.6		10.0	
1 (%)	65.0	52.2		40.0	
2 (%)	25.0	25.1		37.5	
3 (%)	0.0	10.8		10.0	
Analytical data					
Total cholesterol (mg/dl)	148.5 \pm 23.7	153.3 \pm 34.5	0.536	160.7 \pm 41.8	0.156
LDL cholesterol (mg/dl)	79.15 \pm 21.34	83.27 \pm 25.77	0.479	89.90 \pm 32.17	0.182
HDL cholesterol (mg/dl)	50.25 \pm 11.69	43.63 \pm 10.81	0.007	48.45 \pm 12.68	0.597
Non-HDL cholesterol (mg/dl)	98.25 \pm 23.48	109.25 \pm 31.12	0.118	112.22 \pm 34.06	0.105
Triglycerides (mg/dl)	95.50 \pm 40.25	132.10 \pm 83.57	<0.001	111.48 \pm 43.36	0.174
Glycemia (mg/dl)	93.7 \pm 18.30	109.59 \pm 36.99	0.001	113.6 \pm 32.1	0.004
GFR (CKD-EPI) (ml/min/1.73 m ²)	74.77 \pm 14.35	75.87 \pm 19.26	0.799	71.78 \pm 17.04	0.462
Galectin-3 (ng/ml)	7.800 (5.603, 9.373)	7.785 (6.023, 9.760)	0.815	8.485 (6.623, 10.058)	0.355
HS C-reactive protein (mg/L)	2.574 \pm 3.120	4.554 \pm 9.786	0.018	3.913 \pm 5.124	0.215
Calcidiol (ng/ml)	23.66 \pm 9.12	19.49 \pm 8.18	0.025	18.67 \pm 7.06	0.023
FGF-23 (RU/ml)	79.28 \pm 40.22	105.17 \pm 156.61	0.024	86.678 \pm 29.642	0.438
MCP-1 (pg/ml)	114.75 \pm 36.29	143.56 \pm 65.37	0.003	158.322 \pm 72.665	0.003
NT-ProBNP (pg/ml)	199.5 (105.8, 269.3)	167.0 (87.6, 419.0)	0.988	203 (96.4, 464.5)	0.583
Parathormone (pg/ml)	69.45 \pm 30.96	65.52 \pm 31.92	0.587	66.226 \pm 32.436	0.714
Phosphate (mg/dl)	3.28 \pm 0.61	3.25 \pm 1.34	0.874	3.34 \pm 0.48	0.424

ASA, acetylsalicylic acid; CABG, coronary artery by-pass graft; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; CKD-EPI, chronic kidney disease epidemiology collaboration; CRF, cardiovascular risk factors; FGF-23, fibroblast growth factor-23; GFR, glomerular filtration rate; HS, high-sensitivity; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; NT-proBNP, N-terminal probrain natriuretic peptide; PAD, peripheral artery disease. The *p* bold values are statistically significant.

^a Group of age- and sex-matched patients with CRF.

^b Comparison between non-CRF and age- and sex-matched CRF patients.

^c 100% and 97.7% of patients underwent coronary angiography in the non-CRF and CRF groups, respectively.

(HDL)-cholesterol levels were higher in the absence of CRFs (50.3 ± 11.7 mg/dl vs. 43.6 ± 10.8 mg/dl; $p = 0.007$).

The biomarker study showed lower hs-CRP (2.574 ± 3.120 mg/L vs. 4.554 ± 9.786 mg/L; $p = 0.018$) and MCP-1 (114.75 ± 36.29 pg/ml vs. 143.56 ± 65.37 pg/ml; $p = 0.003$) plasma levels in the non-CRF as compared to the CRF group. The analysis of the components of mineral metabolism showed lower FGF-23 levels in patients without CRF as compared to those with CRFs (79.28 ± 40.22 RU/ml vs. 105.17 ± 156.61 RU/ml; $p = 0.024$). On the other hand, calcidiol plasma levels were higher in the non-CRF than in the CRF group (23.66 ± 9.12 ng/ml vs. 19.49 ± 8.18 ng/ml respectively; $p = 0.025$). No differences were found in the remaining biomarkers assessed (Table 1). Using linear regression analysis, we estimated that glycemia, HDL-cholesterol, and triglyceride plasma levels only explained 3.0% ($R^2 = 0.030$), 0.1% ($R^2 = 0.001$), 0.3% ($R^2 = 0.003$), and 6% ($R^2 = 0.062$) of the variations in hs-CRP, MCP-1, FGF-23, and calcidiol levels, respectively.

As the low number of patients without CRFs could have masked significant differences in age and gender, we carried out a new analysis comparing the non-CRF group to a control group of 40 age- and sex-matched patients with CRFs. The results of this analysis were similar to those of the overall assessment (Table 1). There were lower glucose (93.7 ± 18.3 mg/dl vs. 113.6 ± 32.1 mg/dl; $p = 0.004$) and MCP-1 (114.75 ± 36.29 pg/ml vs. 158.32 ± 72.67 pg/ml; $p = 0.003$) plasma levels in the non-CRF group. In addition, calcidiol levels were also higher in the non-CRF than in the CRF group (23.66 ± 9.12 ng/ml vs. 18.67 ± 7.06 ng/ml; $p = 0.023$). There was a trend toward a reduction in FGF-23 and hs-CRP levels in patients without CRF; this difference failed to reach statistical significance, probably due to the lower sample size of both groups in this confirmation study. Similarly, differences in the use of oral antidiabetic drugs, and in triglyceride and HDL plasma levels did not reach statistical significance.

After 2.12 (1.37–2.98) years of follow-up, 2 patients (10%) in the non-CRF group and 75 patients (10.96%) in the CRF group reached the prespecified outcome. Of the 2 patients in the non-CRF group, 1 suffered a stroke and the other developed a non-ST segment elevation myocardial infarction. In the CRF group, there were 38 non-ST segment elevation acute coronary syndromes, 4 cases of ST-segment elevation myocardial infarction, 7 strokes, 10 transitory ischemic attacks, 16 hospital admissions due to heart failure, and 22 deaths. Twelve patients developed two events and five patients experienced three events. There were no significant differences between the two groups ($p = 0.816$, log-rank test).

All variables shown in Table 1 were entered into a multivariate Cox model to investigate the predictive value of the biomarkers studied in the whole population. In this analysis, low calcidiol and high FGF-23 and MCP-1 plasma levels were independent predictors of outcome, along with age, insulin, and angiotensin-converting enzyme inhibitor or angiotensin receptor blocker treatment (Table 2).

Table 2
Multivariable Cox model for the incidence of the outcome.

	Hazard ratio	95% CI		p-Value
		Lower	Upper	
Age	1.054	1.030	1.078	<0.001
Insulin	2.517	1.103	5.746	0.028
ACEI/ARB	0.567	0.336	0.959	0.034
Calcidiol	0.964	0.930	0.999	0.042
MCP-1	1.311 ^a	1.072 ^a	1.604 ^a	0.008
FGF-23	1.112 ^b	1.015 ^b	1.218 ^b	0.023

CI, confidence interval; ACEI, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; MCP-1, monocyte chemoattractant protein-1; FGF-23, fibroblast growth factor-23.

^a Increase in hazard ratio per 100 pg/ml.

^b Increase in hazard ratio per 100 RU/ml.

Discussion

Most patients with established CHD have some classical CRF. Nonetheless, a small proportion of them are free of CRFs. However, little attention has been paid to these patients.

Only 20 out of the 704 patients (2.8%) included in our study had no CRFs. This value is lower than those reported previously [17]. The published data vary depending on the population analyzed, cut-off values used in the definition of CRFs, and age. The absence of traditional CRFs in patients with atherosclerosis is not surprising, since other CRFs may contribute to this disorder [18]. In addition, other factors such as inflammation may promote atherosclerosis [19].

Age is considered an independent CRF [1], and the proportion of patients without classical CRFs increases with age [17], as we observed in our study. Of interest, the prognostic power of classical CRFs diminishes with increasing age [20] suggesting that their role in the development of CHD may be more important in young subjects. Male gender is also correlated with higher cardiovascular risk [21], and in our population there was a higher proportion of men than women. However, we did not find significant differences in gender distribution between patients with and without CRFs, although this could be due to the small number of patients without CRFs.

Both groups showed a similar distribution of clinical variables; the only exception to this was that the CRF group contained more patients under oral antidiabetic therapy. This difference is logical as, by definition, diabetic patients could only belong to the CRF group. Although similar data could be expected to be present with regard to the use of lipid- and blood pressure-lowering drugs, this was not the case. The percentage of patients receiving treatment with statins was similar in both groups, because aggressive lipid lowering is indicated in all patients after an acute coronary event [22]. With regard to antihypertensive patients, modulators of the renin-angiotensin system are indicated in subjects with clinically evident atherosclerosis, and even more when left ventricular dysfunction is present [22]. Similarly, beta-blockers are beneficial in patients with a history of myocardial infarction [22]. Other blood pressure-lowering drugs such as diuretics may be advised in more patients in addition to those with hypertension, such as patients who have experienced heart failure. Finally, patients without CRFs had lower glycemia, higher HDL-cholesterol, and lower triglyceride plasma levels than those with CRFs, according to the criteria used to allocate them into one of the two groups.

Inflammation is an important process both in atherogenesis and plaque complications [19]. Furthermore, some inflammatory markers predict cardiovascular events even in patients without CRFs. In this regard, the ABC study showed that levels of interleukin-6 predicted CHD in elderly patients even in the absence of CRFs [20].

Hs-CRP plasma levels have been associated with an increased incidence of cardiovascular events [2,3]. In our study, patients without CRFs had lower hs-CRP levels than those with classical CRFs. Accordingly, MCP-1 plasma levels were also lower in patients without CRFs. MCP-1 is the most important chemokine involved in the atherothrombotic process, and it is vital to the recruitment of macrophages into the vessel wall [23]. It is expressed more strongly in atherosclerotic lesions than in healthy vessels [24]. Drugs with antiatherosclerotic effects, such as statins, decrease MCP-1 expression in the vascular wall [25]. Furthermore, MCP-1 also has procoagulant properties [26]. According to this evidence, increased MCP-1 plasma levels have been found to predict the development of adverse cardiovascular events in patients with acute coronary syndromes and stable CHD [4,5].

Although traditionally related to renal disease, abnormalities in mineral metabolism have been associated with cardiovascular disease more recently. When there is a decrease in renal

glomerular filtration, phosphate elimination is reduced. In order to maintain phosphate homeostasis, there is an increase in FGF-23 and parathormone plasma levels. Enhanced plasma levels of phosphate, FGF-23, and parathormone have been related to an increase in cardiovascular events through different mechanisms, including endothelial dysfunction, arterial stiffness, and left ventricular hypertrophy [9,10,12,27]. In addition, FGF-23 reduces vitamin D plasma levels [10]. This enhances cardiovascular risk even more, as low vitamin D levels are associated with endothelial dysfunction, inflammation, activation of the renin–angiotensin system, vascular smooth muscle cell proliferation, and vascular calcification, resulting in increased risk of hypertension, CHD, and stroke [11,28–31]. In our population, subjects without CRFs not only exhibited lower FGF-23 plasma levels, but also revealed increased calcidiol levels. This is especially important, since the combination of vitamin D deficit along with increased FGF-23 plasma levels seems to confer a higher cardiovascular risk [32].

It is possible that a causal relationship between the absence of classical CRFs and the more favorable biomarker profile described in this population exists. In this regard, diabetes has been associated with enhanced MCP-1 expression [33] and HDL has been shown to decrease the expression of this cytokine [34]. Furthermore, vitamin D decreases MCP-1 secretion in U937 monocytes in the presence of high glucose concentrations [35]. Finally, FGF-23 decreases synthesis and enhances catabolism of vitamin D [10]. Nevertheless, in the present paper, we have not addressed this issue, and more investigations are needed to elucidate the links between the absence of CRFs and the biomarker profile reported in this paper.

The diminution in hs-CRP, MCP-1, and FGF-23 plasma levels, along with the increase in calcidiol levels appears to correlate with a more favorable prognosis in patients without CRFs. Although this improved prognosis was not confirmed in our population, this could be due to the low number of patients who had no CRFs. Given the low percentage of CHD patients without CRFs, confirmation of a better prognosis would require a large sample size.

However, we show that in our whole population low calcidiol and high MCP-1 and FGF-23 plasma levels were independent predictors of the outcome, suggesting that the biomarker profile on non-CRF patients may be related with a more favorable prognosis.

This work has some limitations. (1) Excluding patients with clinical instability in the first days after the index event could have introduced a certain bias, because these patients would probably have had a worse prognosis, and could have also a different distribution of CRFs. (2) The low number of patients without CRF in our population could have limited the statistical power in the prognosis assessment. This suggests that large sample sizes of patients with CHD must be examined in order to reach more sound conclusions with regard to potential differences in the prognosis of both groups. (3) We have not assessed these biomarkers in the acute phase of the study as, at the time of the acute event, the population was not homogeneous as there were patients with both ST-elevation and non-ST elevation acute coronary syndromes. Then, plasma analysis at this time would imply mixing patients with two different clinical pictures. (4) Finally, it must be emphasized that we have published previously Cox regression analyses in this population to explore separately the predictive power of MCP-1 [5] and that of calcidiol and FGF-23 plasma levels [32]. In the present paper, we have reanalyzed these biomarkers together to support the hypothesis that the biomarker profile of non-CRF patients may be associated with a more favorable prognosis.

In conclusion, patients with CHD who do not present with CRFs display lower plasma levels of inflammatory biomarkers and a better profile in the mineral metabolism than those with CRFs. These findings could be related to a better prognosis, although we

could not confirm this difference given the low number of subjects without CRFs found in our population. Further studies in large numbers of CHD patients are needed in order to confirm this hypothesis.

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Conflict of interest

J. Egido is a lecturer for AbbVie Laboratories. The remaining authors have no conflicts of interest.

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