Original Articles

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

Ana María Pello, MD,[a] Carmen Cristóbal, MD, PhD,[b,c] Nieves Tarín, MD, PhD,[d] Ana Huelmos, MD, PhD,[e] Álvaro Aceña, MD,[a] Rocío Carda, MD,[a] María Luisa González-Casaus, MD,[f] Joaquín Alonso, MD, PhD,[b,c] Óscar Lorenzo, PhD,[g,h] Luis Blanco-Colio, PhD[g], José Luis Martín-Ventura, PhD[g,h], Juan Antonio Franco Peláez, MD, PhD,[a] Ignacio Mahíllo-Fernández, PhD,[i] Jerónimo Farré, MD, PhD,[a,h] Lorenzo López-Bescós, MD, PhD,[c] Jesús Egido, MD, PhD,[g,h,j,k] José Tuñón, MD, PhD,[a,g,h]

[a]Department of Cardiology, IIS-Fundación Jiménez Díaz, Madrid, Spain; [b]Department of Cardiology, Hospital de Fuenlabrada, Fuenlabrada, Spain; [c]Rey Juan Carlos University, Alcorcón, Spain; [d]Department of Cardiology, Hospital Universitario de Móstoles, Spain; [e]Department of Cardiology, Hospital Universitario Fundación Alcorcón, Spain; [f]Laboratory of Nephrology and Mineral Metabolism, Hospital Gómez-Ulla, Madrid, Spain; [g]Laboratory of Vascular Pathology, IIS-Fundación Jiménez Díaz, Madrid, Spain; [h]Autónoma University, Madrid, Spain; [i]Department of Epidemiology, IIS-Fundación Jiménez Díaz, Madrid, Spain; [j]Department of Nephrology, IIS-Fundación Jiménez Díaz; [k]CIBERDEM, Madrid, Spain.

Corresponding author: José Tuñón, MD, Ph.D.**,** Department of Cardiology, IIS-Fundación Jiménez Díaz, Avenida Reyes Católicos 2, 28040 Madrid (Spain), **Phone (+34) 91550480 Ext 3701, email** jtunon@secardiologia.es**, Fax (+34) 915504904**

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ABSTRACT

BACKGROUND

Patients with coronary heart disease (CHD) without classical cardiovascular risk factors (CRF) 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: hs-CRP (high-sensitivity C-reactive protein), MCP-1 (monocyte chemoattractant protein-1), galectin-3, NT-pro-BNP (N-terminal fragment of brain natriuretic peptide), 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 to 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.

INTRODUCTION

The importance of cardiovascular risk factors (CRF) 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 classical CRFs. Although these patients are managed similarly to patients that have CRFs, they sometimes show differences as compared to those with CRFs. In spite of this, less attention has been paid to this subgroup.

In recent years there has been 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 & BAMI (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 on an outpatient basis between six and twelve 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, 1,898 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 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 sudy was carried out during the visit performed six to twelve 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 hypertension if they had a history of systolic and/or diastolic pressure equal to or higher than 140 and 90 mm Hg, 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 (LDL 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 diease.[15]Finally, patients were considered to be diabetics if they were receiving therapy for the disease 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 2,500 g for 10 minutes. 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, Immutopics Inc, San Clemente, CA), intact parathormone was analyzed by a second-generation automated chemiluminescent method (Elecsys 2010 platform, Roche Diagnostics, Mannheim, Germany), and phosphate was determined through an enzymatic method (Integra 400 analyzer, Roche Diagnostics, Mannheim, Germany). Plasma concentrations of MCP-1 and galectin-3 were determined in duplicate using commercially available enzyme-linked immunosorbent assay kits (BMS279/2 Bender MedSystems, and DCP00, R&D Systems, respectively) following the manufacters' 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, Germany), and NT-pro-BNP was analyzed by immunoassay (VITROS, Orthoclinical Diagnostics, U.S.A.). Lipids, glucose, and creatinine determinations were performed by standard methods (ADVIA 2400 Chemistry System, Siemens, Germany).

Statistical analysis

Quantitative data that followed a normal distribution are presented as mean±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 X^2 or Fisher exact test where appropriate. Log-rank test were 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) 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 vs. 109.6 mg/dl; *P*=0.001) and triglycerides (95.5±40.3 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, HDL-cholesterol levels were higher in the absence of CRFs (50.3±11.7 vs. 43.6±10.8 mg/dl; *P*=0.007).

The biomarker study showed lower hs-CRP (2.574±3.120 vs 4.554±9.786 mg/L; *P*=0.018) and MCP-1 (114.75±36.29 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 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 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 vs. 113.6±32.1 mg/dl; *P*=0.004) and MCP-1 (114.75±36.29 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 vs. 18.67±7.06 ng/ml; *P*=0.023). There was a trend towards 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 5 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 inhibitors or angiotensin receptor blockers treatment (Table 2).

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, cutoff values used in the definition of CRFs, and age. 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. Male gender is also correlated with higher cardiovascular risk,[18] 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.[19] 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.[19] Similarly, beta-blockers are beneficial in patients with a history of myocardial infarction.[19] Other blood pressure lowering drugs such as diuretics may be adviced 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 complication.[20] In this regard, 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.[21] It is expressed more strongly in atherosclerotic lesions than in the healthy vessel.[22] Drugs with antiatherosclerotic effects, such as statins, decrease MCP-1 expression in the vascular wall.[23] Furthermore, MCP-1 also has procoagulant properties.[24] 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 mantain phospate

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.[9,10,12,25] 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.[11,26-29] 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.[30]

The diminution in hs-CRP, MCP-1, and FGF-23 plasma levels, along with the increase in calcidiol levels seems 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 that had no CRFs. Given the very low percentage of CHD patients without CRFs, confirmation of a better prognosis would require a very large sample size.

However, we show than 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. 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. The low number of patients without CRF in our population could have limited the statistical power in the prognosis assessment. This suggests that very 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. 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 [30]. 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 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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REFERENCES

[1]. Ridker PM, Libby P. Risk markers for atherothrombotic disease. In: Bonow RO, Mann DL, Zipes DP, Libby P, eds. Braunwald's Heart Disease. A textbook in cardiovascular Medicine (ninth edition). Elsevier, Philadelphia 2012:914-34.

[2]. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation 1998;97:2007-11.

[3]. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.

[4]. de Lemos JA, Morrow DA, Sabatine MS, Murphy SA, Gibson CM, Antman EM, McCabe CH, Cannon CP, Braunwald E. Association between plasma levels of monocyte chemoattractant protein-1 and long-term clinical outcomes in patients with acute coronary syndromes. Circulation 2003;107:690-5.

[5]. Tuñón J, Blanco-Colio L, Cristóbal C, Tarín N, Higueras J, Huelmos A, Alonso J, Egido J, Asensio D, Lorenzo O, Mahíllo-Fernández I, Rodríguez-Artalejo F, Farré J, Martín-Ventura JL, López-Bescós L. Usefulness of a combination of monocyte chemoattractant protein-1, galectin-3, and N-terminal probrain natriuretic Peptide to predict cardiovascular events in patients with coronary artery disease. Am J Cardiol 2014;113:434-40.

[6]. Di Angelantonio E, Chowdhury R, Sarwar N, Ray KK, Gobin R, Saleheen D, Thompson A, Gudnason V, Sattar N, Danesh J. Type Natriuretic Peptides and Cardiovascular Risk: Systematic Review and Meta-Analysis of 40 Prospective Studies. Circulation 2009;120:2177-87.

[7]. Lok DJ, Van Der Meer P, de la Porte PW, Lipsic E, Van Wijngaarden J, Hillege HL, van Veldhuisen DJ. Prognostic value of galectin-3, a novel marker of fibrosis, in patients with chronic heart failure: data from the DEAL-HF study. Clin Res Cardiol 2010;99:323-8.

[8]. Dumic J, Dabelic S, Flögel M. Galectin-3: an open-ended story. Biochim Biophys Acta 2006;1760:616-35.

[9]. Ketteler M, Wolf M, Hahn K, Ritz E. Phosphate: a novel cardiovascular risk factor. Eur Heart J 2013;34:1099-101.

[10]. Wolf M. Forging forward with 10 burning questions on FGF23 in kidney disease. J Am Soc Nephrol 2010;21:1427-35.

[11]. Lavie CJ, Lee JH, Milani RV. Vitamin D and cardiovascular disease will it live up to its hype? J Am Coll Cardiol 2011;58:1547-56.

[12]. van Ballegooijen AJ, Reinders I, Visser M, Brouwer IA. Parathyroid hormone and cardiovascular disease events: A systematic review and meta-analysis of prospective studies. Am Heart J. 2013 May;165:655-64.

[13]. Mancia G, Fagard R, Narkiewicz K, Redon J, ZAnchetti A, Böhm M, Christiaens T, Cifkova R, De Baer G, Dominiczak A, Galderisi M, Grobbee DE, Jaarsma T, Kirchhof P, Kjeldsen SE, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2013;34:2159-219.

[14]. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). JAMA 2001;285:2486-97.

[15]. Otaki Y, Gransar H, Berman DS, Cheng VY, Dey D, Lin FY, Achenbach S, Al-Mallah M, Budoff MJ, Cademartiri F, Callister TQ, Chang HJ, Chinnaiyan K,Chow BJ, Delago A, et al. Impact of family history of coronary artery disease in young individuals (from the CONFIRM registry). Am J Cardiol 2013;111:1081–6.

[16]. Rydén L, Grant PJ, Anker SD, Berne C, Cosentino F, Danchin N, Deaton C, Escaned J, Hammes HP, Huikuri H, Marre M, Marx N, Mellbin L, Ostergren J, Patrono C, et al. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD: the Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). Eur Heart J 2013;34:3035-87.

[17]. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, Ellis SG, Lincoff Am, Topol EJ. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 2003;290:898-904.

[18]. Dawber TR, Moore FE, Mann GV. Coronary heart disease in the Framingham study. Am J Public Health Nations Health 1957;47:4-24.

[19]. Montalescot G, Sechtem U, Achenbach S, Andreotti F, Arden C, Budaj A, Bugiardini R, Crea F, Cuisset T, Di Mario C, Ferreira JR, Gersh BJ, Gitt AK, Hulot JS, Marx N, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the Task Force on the management of stable coronary artery disease of the European Society of Cardiology. Eur Heart J. 2013;34:2949-3003.

[20]. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1999;340:115-26

[21]. Inoue S, Egashira K, Ni W, Kitamoto S, Usui M, Otani K, Ishibashi M, Hiasa K, Nishida K, Takeshita A . Anti-monocyte chemoattractant protein-1 gene therapy limits progression and destabilization of established atherosclerosis in apolipoprotein E-knockout mice. Circulation 2002;106:2700-6.

[22]. Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest 1991;88:1121-7.

[23]. Bustos C, Hernández-Presa MA, Ortego M, Tuñón J, Ortega L, Pérez F, Díaz C, Hernández G, Egido J. HMG-CoA reductase inhibition by atorvastatin reduces neointimal inflammation in a rabbit model of atherosclerosis. J Am Coll Cardiol 1998;32:2057-64.

[24]. Schecter AD, Rollins BJ, Zhang YJ, Charo IF, Fallon JT, Rossikhina M, Giesen PL, Nemerson Y, Taubman MB. Tissue factor is induced by monocyte chemoattractant protein-1 in human aortic smooth muscle and THP-1 cells. J Biol Chem 1997;272:28568-673.

[25]. Parker BD, Schurgers LJ, Brandenburg VM, Christenson RH, Vermeer C, Ketteler M, Shlipak MG, Whooley MA, Ix JH. The associations of fibroblast growth factor 23 and uncarboxylated matrix Gla protein with mortality in coronary artery disease: the Heart and Soul Study. Ann Intern Med 2010;152:640-8.

[26]. Scragg R, Jackson R, Holdaway IM, Lim T, Beaglehole R. Myocardial infarction is inversely associated with plasma 25-hydroxyvitamin D3 levels: a community-based study. Int J Epidemiol 1990;19:559-63.

[27]. Syal SK, Kapoor A, Bhatia E, Sinha A, Kumar S, Tewari S, Garg N, Goel PK. Vitamin D deficiency, coronary artery disease, and endothelial dysfunction: observations from a coronary angiographic study in Indian patients. J Invasive Cardiol 2012;24:385-9.

[28]. Watson KE, Abrolat ML, Malone LL, Hoeg JM, Doherty T, Detrano R, Demer LL. Active serum vitamin D levels are inversely correlated with coronary calcification. Circulation 1997;96:1755-60.

[29]. Gonzalez-Parra E, Rojas-Rivera J, Tuñón J, Praga M, Ortiz A, Egido J. Vitamin D receptor activation and cardiovascular disease. Nephrol Dial Transplant 2012;27:17-21.

[30]. Tuñón J, Cristóbal C, Tarín N, Aceña Á, González-Casaus ML, Huelmos A, Alonso J, Lorenzo Ó, González-Parra E, Mahíllo-Fernández I, Pello AM, Carda R, Farré J, Rodríguez-Artalejo F, López-Bescós L et al. Coexistence of low vitamin d and high fibroblast growth factor-23 plasma levels predicts an adverse outcome in patients with coronary artery disease. PLoS One. 2014 Apr 18;9:e95402