



Revista de Osteoporosis y Metabolismo Mineral

ISSN: 1889-836X

ISSN: 2173-2345

Sociedad Española de Investigaciones Óseas y
Metabolismo Mineral

Gómez Alonso, C; Rodríguez García, M; Avello Llano, N; García Gil-Albert,
C; Palomo Antequera, C; Fernández Villabrille, S; Rodríguez Carrio, J; Díaz
Sottolano, AA; Fernández Martín, JL; Cannata Andía, JB; Naves Díaz, M
Asociación de parámetros bioquímicos del metabolismo óseo
con progresión y/o desarrollo de nuevas calcificaciones aórticas
Revista de Osteoporosis y Metabolismo Mineral, vol. 13, no. 1, 2021, pp. 10-16
Sociedad Española de Investigaciones Óseas y Metabolismo Mineral

DOI: <https://doi.org/10.4321/S1889-836X2021000100003>

Available in: <https://www.redalyc.org/articulo.oa?id=360966808003>

- How to cite
- Complete issue
- More information about this article
- Journal's webpage in redalyc.org

redalyc.org

Scientific Information System Redalyc

Network of Scientific Journals from Latin America and the Caribbean, Spain and
Portugal

Project academic non-profit, developed under the open access initiative

Association of biochemical parameters of bone metabolism with progression and/or development of new aortic calcifications

DOI: <http://dx.doi.org/10.4321/S1889-836X2021000100003>

Gómez Alonso C¹, Rodríguez García M², Avello Llano N³, García Gil-Albert C³, Palomo Antequera C⁴, Fernández Villabrille S¹, Rodríguez Carrio J⁵, Díaz Sottolano AA⁶, Fernández Martín JL¹, Cannata Andía JB¹, Naves Díaz M¹

¹ Bone Metabolism Clinical Management Unit. Central University Hospital of Asturias. University of Oviedo, Institute of Health Research of the Principality of Asturias (ISPA). Renal Research Network of the Carlos III Health Institute (REDinREN of the ISCIII). Oviedo (Spain)

² Nephrology Clinical Management Area. Central University Hospital of Asturias. University of Oviedo, ISPA. REDinREN of the ISCIII. Oviedo (Spain)

³ Laboratory of Medicine. Central University Hospital of Asturias. Oviedo (Spain)

⁴ Unit of Clinical Management of Internal Medicine. Central University Hospital of Asturias. University of Oviedo, ISPA. Oviedo (Spain)

⁵ Basic and Translational Research in Chronic Inflammatory Diseases. University of Oviedo, ISPA. Oviedo (Spain)

⁶ Hospital de Cabueñes, ISPA. Gijón (Spain)

Date of receipt: 13/12/2020 - Date of acceptance: 07/02/2021

Summary

Objective: Biochemical parameters continue to be the most widely used option for the follow-up of patients with bone metabolic disorders. The objective of our study was to assess the association of some biochemical markers of bone metabolism with the appearance and progression of aortic calcifications.

Material and methods: In this study, 624 men and women older than 50 years were selected at random. The participants completed a questionnaire and underwent two lateral dorsal-lumbar x-rays and bone densitometry. Four years later, the same studies were repeated in 402 subjects along with a biochemical study.

Results: Age and the proportion of men were higher in those who had "global progression" of aortic calcification (progression of the existing ones plus new ones). The serum levels of calcium and calcitriol were significantly higher and those of osteocalcin significantly lower in which "global progression" of aortic calcification was observed. Multivariate analysis showed that only osteocalcin was independently associated with "global progression" of aortic calcification, with an 18% decrease for each 1 ng/mL increase in osteocalcin levels (odds ratio (OR)=0.82; 95% confidence interval (95% CI): 0.71-0.92). The categorization of osteocalcin into tertiles showed that the subjects of the first tertile (<4.84 ng/mL) were associated with a higher proportion of new aortic calcifications: (OR=2.45; 95% CI: 1.03-3.56) with respect to the third tertile (>6.40 ng/mL).

Conclusion: Serum levels of osteocalcin could be a biochemical marker to evaluate the appearance and/or evolution of aortic calcification. However, it is necessary to determine with greater precision how it could exert this protective effect in the process of vascular calcification.

Key words: osteocalcin, vascular calcification, biochemical markers, bone mineral density.

INTRODUCTION

Atherosclerosis, arteriosclerosis, vascular calcification and osteoporosis are common age-related disorders associated with high morbidity and mortality^{1,2}. Due to the increased life expectancy in the Spanish population, these disorders are expected to become more and more frequent in the coming decades. Although recent work has been carried out on the development of non-invasive techniques for the early detection of vascular calcifications, such as pulse wave velocity and non-contrast carotid ul-

trasound, serum biochemical parameters continue to be the most widely used option for monitoring patients with bone metabolic disorders³⁻⁵.

Having easily accessible non-invasive tools such as biochemical markers allow for the adoption of therapeutic measures in order to mitigate the deleterious effect of bone loss. Taking into account that osteoporosis and vascular calcification share etiopathogenic mechanisms^{6,7}, some biochemical parameters used to study bone metabolism could serve as possible markers of vascular cal-



Correspondence: Manuel Naves Díaz (mnaves.huca@gmail.com)

cification. Therefore, this study aims to assess the association of some biochemical markers of bone metabolism with the appearance and progression of aortic calcifications.

MATERIAL AND METHODS

This study used data from a European project designed to determine the prevalence of vertebral fracture (European Vertebral Osteoporosis Study - EVOS)⁸, in which the Bone and Mineral Metabolism Service of the Central University Hospital of Asturias took part.

For this work, 308 men and 316 women over 50 years of age were selected at random from the municipal registry of Oviedo. Our protocol involved patients' filling out a questionnaire on risk factors related to osteoporosis, two lateral dorsal-lumbar x-rays and a bone densitometry (DXA) (the radiographic study was not completed in only 2 cases), and collecting anthropometric measurements such as height and weight to determine body mass index (BMI). All subjects had sufficient stamina to go up two floors without an elevator and 99% lived in their own home.

After four years, they were invited to repeat the radiological study, bone densitometry, anthropometric measurements and respond to a questionnaire on risk factors for osteoporosis and a biochemical study. In the second control, 402 subjects participated (213 women and 189 men), of whom 335 agreed to carry out the biochemical study. A total of 67 subjects (16.7%) were excluded from the analysis because they had been treated for osteoporosis or their renal function was impaired with serum creatinine greater than 0.8 mg/dL in women and 1.1 mg/dL in men, respectively. All data were available at baseline and at 4 years in 262 subjects.

Evaluation of the progression of vascular calcification

Abdominal aortic calcification was evaluated by two independent investigators, and was defined and classified as grade 0 (absent), grade 1 (mild-moderate), and grade 2 (severe). Isolated punctate calcifications, a visible linear calcification in less than 2 vertebral bodies, or a dense calcified plaque were defined as mild-moderate calcification⁹. The presence of a visible linear calcification along at least two vertebral bodies and/or the presence of two or more calcified dense plaques was defined as severe calcification. The degree of intra- and inter-observer concordance in the analysis of the x-rays was 92% and 90%, respectively, with a Kappa coefficient of 0.78 and 0.73, data that indicate good reproducibility⁹.

The progression of aortic calcification was determined by comparing the x-rays taken at baseline with those at 4 years. It was defined as "global progression" of aortic calcification when an increase in the magnitude of baseline aortic calcification coexisted with the appearance of new calcifications, comparing the x-rays at the outset with those done 4 years later.

Densitometric evaluation

Bone mineral density (BMD) was measured with a Hologic® QDR-1000 DXA densitometer (Hologic Inc., Waltham, Massachusetts, USA). In all cases, the anteroposterior lumbar spine (L2-L4) and the proximal extremity of the right femur were analyzed. For the evaluation of lumbar BMD, 4 subjects with marked degenerative osteoarthritis were excluded. The coefficients of variation (CV) were

1.2% and 1.9%, respectively⁹. The precision and quality control was performed daily with a lumbar spine phantom, with which a CV of $0.0 \pm 0.1\%$ was obtained. In the fourth year, BMD was determined in the same areas used in the first study, and the percentage of change between both measurements was used to evaluate changes in BMD.

Biochemical analysis

In the baseline study, no biochemical study was carried out. At 4 years, a fasting blood and urine sample was taken from each subject participating in the study. Once the serum was separated, the latter and the urine were kept frozen at -80°C until quantification. Serum calcium, creatinine, phosphorus, total alkaline phosphatase, and acid resistant tartrate phosphatase were measured using an autoanalyzer (Hitachi Mod. 717, Ratigen, Germany). The serum levels of calcidiol (25OHD) were determined by prior extraction with acetonitrile (IDS, Ltd., Bolton, United Kingdom), whose intra- and inter-assay coefficients of variation (CV) were 5.2% and 8.2%, respectively.

Levels of 1,25-dihydroxyvitamin D were measured by radioimmunoassay (IDS, Ltd.); intra- and interassay CVs were 6.5% and 9%, respectively. The intact PTH and total osteocalcin levels were measured by radioimmunoassay (Nichols Institute, San Juan Capistrano, California, USA). Intra- and inter-assay CV values were 2.6% and 5.8% for PTH and 4.5% and 5.1% for osteocalcin, respectively.

All the tests carried out followed the principles set forth in the Declaration of Helsinki and were formally approved by the Committee for Clinical Trials of the Principality of Asturias.

Statistic analysis

Data analysis was carried out using SPSS version 17.0 for Windows. The quantitative variables were analyzed by Student's t test and the qualitative variables by chi-square.

Multivariate analysis was performed using logistic regression adjusting for age, sex and BMI, in those serum or urinary markers in which the univariate analysis was significantly associated with progression and/or appearance of new abdominal aortic calcification.

Pearson correlations were performed between those biochemical parameters that, at the multivariate level, showed a significant association with the percentage of change in BMD between both cross-sectional studies.

RESULTS

The mean age of those who had "global progression" of aortic calcification (progression of existing vascular calcifications plus new vascular calcifications) was higher than the age of those in whom this situation was not observed (Tables 1 and 2). However, there were no age differences in those who only presented a new aortic calcification as a change in the control at 4 years (Table 3). The BMI was similar in those with aortic calcification, both in those in which the calcification progressed, as in those with new calcifications, or considering both variations together (Tables 1-3).

Male sex was significantly more frequent in those in whom progression of existing aortic calcifications and/or new aortic calcifications was observed. In contrast, there were no differences in the smoking habit (Tables 1-3).

Table 1. Clinical and anthropometric variables and biochemical markers of bone and mineral metabolism in the presence or absence of “global progression” of vascular calcification (CV)

Progression more new CV	Aortic calcification (n=118)	No aortic calcification (n=144)	P value
Male gender	77 (72.3%)	61 (42.4%)	<0.001
Smoker	23 (19.5%)	18 (12.5%)	0.121
Age (years)	69.6 ± 7.7	66.4 ± 8.9	0.002
BMI (kg/cm ²)	28.0 ± 3.8	28.4 ± 4.2	0.367
PTH (pg/mL)	54.0 ± 27.1	51.8 ± 20.5	0.460
Alkaline phosphatase (IU/L)	177 ± 89	175 ± 55	0.817
Calcium (mg/dL)	9.46 ± 0.30	9.35 ± 0.34	0.011
Phosphorus (mg/dL)	3.45 ± 0.44	3.44 ± 0.48	0.964
Calcidiol (ng/mL)	15.5 ± 7.5	17.4 ± 9.8	0.092
Calcitriol (pg/mL)	43.9 ± 17.3	39.4 ± 14.4	0.025
Osteocalcin (ng/mL)	5.42 ± 1.76	6.22 ± 2.15	0.002
FATR (U/L)	2.02 ± 0.65	2.13 ± 0.64	0.212
Ca/creatinine urine	0.18 ± 0.11	0.17 ± 0.10	0.675

The variables are expressed in number (percentage) and in mean ± standard deviation; BMI: body mass index; PTH: parathormone; FATR: tartrate-resistant acid phosphatase.

Table 2. Clinical and anthropometric variables and biochemical markers of bone and mineral metabolism in the presence or absence of progression of vascular calcifications (VC)

CV progression	Aortic calcification (n=62)	No aortic calcification (n=144)	P value
Male gender	40 (64.5%)	61 (42.4%)	0.003
Smoker	13 (21.0%)	18 (12.5%)	0.119
Age (years)	70.6 ± 8.2	66.4 ± 8.9	0.002
BMI (kg/cm ²)	27.8 ± 4.0	28.4 ± 4.2	0.319
PTH (pg/mL)	54.7 ± 29.2	51.8 ± 20.5	0.428
Alkaline phosphatase (IU/L)	175 ± 51	175 ± 55	1.000
Calcium (mg/dL)	9.48 ± 0.26	9.35 ± 0.34	0.009
Phosphorus (mg/dL)	3.42 ± 0.45	3.44 ± 0.48	0.774
Calcidiol (ng/mL)	14.8 ± 7.6	17.4 ± 9.8	0.082
Calcitriol (pg/mL)	42.8 ± 17.2	39.4 ± 14.4	0.154
Osteocalcin (ng/mL)	5.46 ± 1.87	6.22 ± 2.15	0.019
FATR (U/L)	2.09 ± 0.58	2.13 ± 0.64	0.692
Ca/creatinine urine	0.17 ± 0.10	0.17 ± 0.10	0.891

The variables are expressed in number (percentage) and in mean ± standard deviation; BMI: body mass index; PTH: parathormone; FATR: tartrate-resistant acid phosphatase.

Regarding the biochemical markers of bone metabolism, serum levels of calcium and calcitriol were significantly higher and those of osteocalcin significantly lower in those subjects in whom “global progression” of aortic calcification was observed (new calcifications plus progression of vascular calcification) (Table 1).

The logistic regression analysis adjusted for age, sex and BMI showed that the only biochemical marker that was independently associated with “global progression” of aortic calcification was osteocalcin, showing that increases of 1 ng/mL were associated with a decrease in 18% in the pro-

gression of aortic calcification (odds ratio (OR)=0.82; 95% confidence interval (95% CI): 0.71-0.95) (Table 4). Age and male sex were also significantly associated with progression of vascular calcification (OR=1.05; 95% CI: 1.01-1.08 and OR=2.06; 95% CI: 1.20-3.54, respectively) (Table 4).

In the univariate analysis, serum osteocalcin levels were significantly lower and calcium levels significantly higher in those subjects in whom aortic calcification had progressed (Table 2). The logistic regression analysis adjusted for age, sex and BMI confirmed that osteocalcin was the only parameter that showed a significant association:

Table 3. Clinical and anthropometric variables and biochemical markers of bone and mineral metabolism in the presence or absence of new vascular calcifications (VC)

New CV	Aortic calcification (n=56)	No aortic calcification (n=144)	P value
Male gender	37 (66.1%)	61 (42.4%)	0.004
Smoker	10 (17.9%)	18 (15.0%)	0.327
Age (years)	68.5 ± 7.1	66.4 ± 8.9	0.082
BMI (kg/cm ²)	28.2 ± 3.6	28.4 ± 4.2	0.691
PTH (pg/mL)	53.3 ± 24.8	51.8 ± 20.5	0.676
Alkaline phosphatase (IU/L)	180 ± 119	175 ± 55	0.717
Calcium (mg/dL)	9.43 ± 0.34	9.35 ± 0.34	0.166
Phosphorus (mg/dL)	3.47 ± 0.44	3.44 ± 0.48	0.694
Calcidiol (ng/mL)	16.3 ± 7.3	17.4 ± 9.8	0.463
Calcitriol (pg/mL)	45.2 ± 17.4	39.4 ± 14.4	0.022
Osteocalcin (ng/mL)	5.36 ± 1.65	6.22 ± 2.15	0.009
FATR (U/L)	1.95 ± 0.73	2.13 ± 0.64	0.103
Ca/creatinine urine	0.18 ± 0.11	0.17 ± 0.10	0.579

The variables are expressed in number (percentage) and in mean ± standard deviation; BMI: body mass index; PTH: parathormone; FATR: tartrate-resistant acid phosphatase.

increases of 1 ng/mL were associated with a 16% increase in the progression of aortic calcifications (OR=0.84; 95% CI: 0.70-0.99) (Table 4). Sex (OR=1.95; 95% CI: 1.01-3.76) and also age (OR=1.06; 95% CI: 1.02-1.10) were associated in this multivariate model (Table 4).

When only those subjects who presented a new aortic calcification were analyzed, serum levels of osteocalcin were found to be significantly lower and those of calcitriol, significantly higher (Table 3). The logistic regression analysis adjusted for age, sex and BMI confirmed that only osteocalcin showed a significant association: increases of 1 ng/mL were associated with a 20% appearance of new aortic calcifications (OR=0.80; 95% CI: 0.67-0.97) (Table 4). Male sex (OR=2.30; 95% CI: 1.15-4.59), but not age, was associated in this multivariate model (Table 4).

The categorization of serum osteocalcin levels into tertiles showed that the lowest tertile (osteocalcin <4.84 ng/mL) was the one that showed the highest proportion of new aortic calcifications (22; 42.3%). The second tertile (osteocalcin between 4.84 and 6.40 ng/mL) showed the same trend, but in a lower proportion (18; 34.6%), while the third tertile (osteocalcin >6.4 ng/mL) showed the lowest proportion (12; 23.1%). A logistic regression analysis adjusted for age, sex and BMI showed that the subjects of the first tertile were associated with a higher proportion (2.45 times) of new aortic calcifications: (OR=2.45; 95% CI: 1.03-3.56). There were no differences with those of the second tertile (OR=1.48; 95% CI: 0.611-3.56).

The bi-variate correlations between the percentage of change in BMD at the lumbar and femoral neck level and the serum levels of osteocalcin showed a negative and significant correlation. Higher values of osteocalcin were associated with a lower loss of BMD, while lower values of osteocalcin were associated with greater losses of bone mass (Figure 1A and 1B).

DISCUSSION

Our results confirm that, of the biochemical markers analyzed, osteocalcin was the only marker associated with the appearance and progression of aortic calcifications independently of age, sex and BMI. A 1 ng/mL increase in osteocalcin decreased the “global progression” of aortic calcification by 18%, a protection equivalent to being 3-4 years younger.

Osteocalcin, a vitamin K-dependent protein, is the most abundant non-collagen component in the mineralized matrix of bone. It is not only produced by bone, but also by vascular smooth muscle cells that show a phenotype similar to osteoblasts¹⁰. It inhibits the precipitation of calcium phosphate and shows a strong affinity for hydroxyapatite¹¹. Initially, it was thought that osteocalcin inhibited the growth of hydroxyapatite crystals¹² and limited bone formation¹³.

Experimental studies have shown that decarboxylated osteocalcin can up-regulate nitric oxide synthesis in human endothelial cells with a protective effect against endothelial dysfunction. These findings support the opinion that decarboxylated osteocalcin is the biologically active form of the protein, with a protective function on the vasculature independent of its metabolic role, although more studies are required to confirm this fact¹⁴.

Osteocalcin has been detected to a greater degree in calcified plaques and aortic valves than in healthy non-calcified vessels^{15,16}. The level of osteocalcin mRNA reportedly increases between 8 and 14 times in calcified aortic plaques compared to healthy aortas¹⁷. The increase in total osteocalcin may occur as a result from the development of an osteogenic phenotype in atherosclerotic plaques¹⁸. However, this requires further validation. Recently, osteocalcin has been found to play a crucial role in arterial calcification mediated by Wnt/ β -catenin signaling through increased oxidative phosphorylation, and this finding may have clinical implications¹⁹.

Table 4. Multivariate analysis of the independent variables significantly associated in the univariate analysis with the progression and/or presence of new aortic calcifications. The odd ratio (OR) and the 95 confidence interval (95% CI) are represented. Significant values are shown in bold

Dependent variable	Independent variables	OR	IC 95%	P value
Global progression of calcification (progression and new)	Age (every year)	1.05	1.01 - 1.05	0.007
	Gender (male)	2.06	1.20 - 3.54	0.009
	Calcium (each mg/dL)	1.87	0.79 - 4.42	0.152
	Calcitriol (each pg/mL)	1.02	0.99 - 1.03	0.068
	Osteocalcin (each ng/mL)	0.82	0.71 - 0.95	0.007
Progression of aortic calcification	Age (every year)	1.06	1.02 - 1.10	0.005
	Gender (male)	1.95	1.01 - 3.76	0.046
	Calcium (each mg/dL)	2.61	0.90 - 7.55	0.077
	Osteocalcin (each ng/mL)	0.84	0.70 - 0.99	0.040
New aortic calcifications	Age (every year)	1.02	0.98 - 1.07	0.250
	Gender (male)	2.30	1.15 - 4.59	0.018
	Calcium (each mg/dL)	1.51	0.56 - 4.03	0.415
	Calcitriol (each pg/mL)	1.02	0.99 - 1.04	0.073
	Osteocalcin (each ng/mL)	0.80	0.67 - 0.97	0.024

Significant values are shown in bold.

However, studies in patients are inconclusive. A relatively recent meta-analysis included 46 studies that examined the association between osteocalcin and atherosclerosis²⁰. Of the studies that analyzed the association between osteocalcin and carotid intima-media thickness (CIMT), four reported that higher levels of osteocalcin were associated with greater CIMT, four reported that higher levels of osteocalcin were associated with a lower CIMT, and three did not find any correlation. However, studies that examined mononuclear cells positive for osteocalcin or histological staining for osteocalcin showed that higher levels of osteocalcin were associated with an increase in markers of atherosclerosis and calcification²⁰. Thus, it is suggested that osteocalcin could be a marker of the calcification process.

Our results show that, in the 4-year period between both cross-sections, both the presence of new aortic calcifications and their progression were associated with lower levels of osteocalcin, regardless of age, sex and BMI. It is noteworthy that the lowest tertile of osteocalcin (<4.84 ng/mL) was associated with a significant increase in new aortic calcifications: 2.45 (1.03-3.56) compared to subjects with serum osteocalcin levels higher than 6.4 ng/mL.

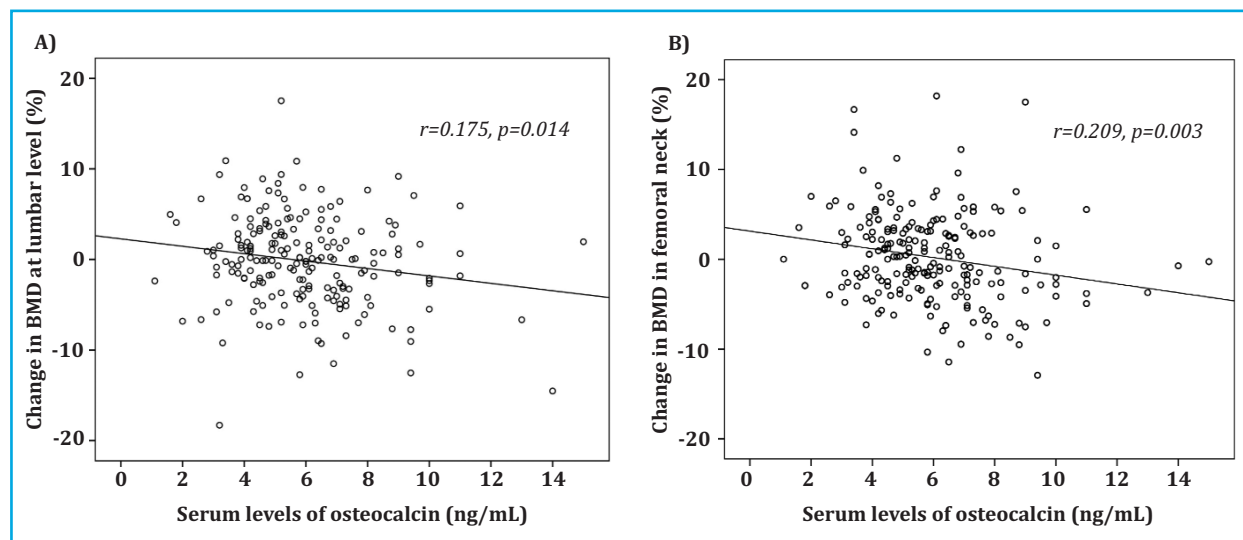
Kim et al. found similar results in Asian women to those in our study, with an inverse correlation between osteocalcin and vascular calcification measured by the Agatston score, even after adjusting for age²¹. Similar results have also been shown in other cross-sectional^{22,23} and longitudinal studies, such as ours, in which raised levels of osteocalcin are found to be associated with less progression of abdominal aortic calcification²⁴. These

authors suggest that osteocalcin could be involved in the aortic calcification process indirectly by its action on insulin and insulin resistance. Fusaro et al. have recently observed in a population on dialysis that those diabetic patients with a higher prevalence of vascular calcification had lower serum levels of total and decarboxylated osteocalcin²⁵. In fact, in a secondary analysis of our study, analyzing osteocalcin levels in those subjects diagnosed with diabetes, it was observed that the presence of diabetes (n=23) was associated with significantly lower levels of osteocalcin than those without diabetes (n=241) (4.89±1.80 ng/mL compared to 5.96±2.14 ng/mL; p=0.020).

It could also be conjectured that low levels of osteocalcin are associated with vascular calcification (VC) due to less bone remodeling, which could be a VC risk factor^{26,27}. However, this possibility would not be supported by the results of this study, since the subjects with lower levels of osteocalcin and higher VC were those with lower BMD, which would be more indicative of high remodeling than low remodeling²⁸.

On the other hand, the usefulness of osteocalcin as a serum marker remains controversial. There is still a long way to go to define whether osteocalcin can be used as a diagnostic or detection tool in the appearance of VC. It is noteworthy that no study has differentiated between forms of osteocalcin when it comes to VC. Consequently, it is necessary to study the effect that carboxylated and decarboxylated osteocalcin could have in this environment, as well as to consider the mechanisms associated with the increase of osteocalcin in calcified tissue⁵.

Figure 1. Bi-variate correlations between changes in BMD in percentage A) at lumbar level and B) femoral neck with serum levels of osteocalcin



This study presents several limitations. First, osteocalcin determination was only carried out in the second cross section, which limits the associations found. Second, intact or total osteocalcin was determined without differentiating between carboxylated or decarboxylated. On the other hand, the evaluation of vascular calcification was carried out by simple X-ray imaging and not by more sensitive techniques. It is also possible that some of the people who attended the second check-up after 4 years would have done so because they were in a worse physical condition compared to those who did not attend it, although no clear selection biases were found²⁹.

Despite these limitations, the study also has important strengths, such as the adequate response of the subjects who participated in the study, both at baseline (50%)³⁰ and at 4 years of the follow-up period (70%). The degree of reliability among observers for the assessment of vascular calcification supports its use as a diagnostic criterion. Finally, unlike other studies, this study was prospective, and not cross-sectional like most of those cited. This reinforces the validity of the results found and their greater degree of association.

Thus, although new studies are needed to confirm these results, this study seems to indicate that serum le-

vels of osteocalcin could be a promising biochemical marker associated with the appearance and/or development of aortic calcification.

Acknowledgments: This research study has been partially funded by the European Study on Vertebral Osteoporosis (EVOS), European Union (1991-1993); European Prospective Osteoporosis Study (EPOS), European Union (BIOMED 93-95), BMHI-CT 092-0182 (1993-1997); Health Research Fund (FIS 94/1901-E); Retic REDinREN of ISCIII (RD06/0016/1013, RD12/0021/0023 and RD16/0009/0017); National R + D + I Plan 2008-2011, State R + D + I Plan 2013-2016, European Regional Development Fund (ERDF), Science, Technology and Innovation Plan 2013-2017 and 2018-2022 of the Principality of Asturias (GRUPIN14-028, IDI-2018-000152), Renal Foundation Íñigo Álvarez de Toledo (FRIAT). Augusto Antonio Díaz Sottolano has been funded by the Principality of Asturias Health Research Institute (ISPA). Sara Fernández Villabrille has been financed by IDI-2018-000152 and project FIS 17/00715 and Javier Rodríguez Carrio by a contract along with Juan de la Cierva and Sara Borrell.



Conflict of interests: The authors declare no conflict of interest.

Bibliography

1. Wilson PW, Kauppila LI, O'Donnell CJ, Kiel DP, Hannan M, Polak JM, et al. Abdominal aortic calcific deposits are an important predictor of vascular morbidity and mortality. *Circulation*. 2001; 103(11):1529-34.
2. Cummings SR, Melton LJ. Epidemiology and outcomes of osteoporotic fractures. *Lancet*. 2002;359(9319):1761-7.
3. Torii S, Arima H, Ohkubo T, Fujiyoshi A, Kadota A, Takashima N, et al. Association between pulse wave velocity and coronary artery calcification in Japanese men. *J Atheroscler Thromb*. 2015; 22(12):1266-77.
4. Zhang H, Du J, Wang H, Wang H, Jiang J, Zhao J, et al. Comparison of diagnostic values of ultrasound micro-flow imaging and contrast-enhanced ultrasound for neovascularization in carotid plaques. *Exp Ther Med*. 2017;14(1):680-8.
5. Arroyo D, Betriu A, Martínez-Alonso M, Vidal T, Valdivielso JM, Fernández E. Observational multicenter study to evaluate the prevalence and prognosis of subclinical atheromatosis in a Spanish chronic kidney disease cohort: baseline data from the NEFRONA study. *BMC Nephrol*. 2014;15:168.
6. Kiel DP, Kauppila LI, Cupples LA, Hannan MT, O'Donnell CJ, Wilson PW. Bone loss and the progression of abdominal aortic calcification over a 25 year period: the Framingham Heart Study. *Calcif Tissue Int*. 2001;68(5):271-6.
7. Schulz E, Arfai K, Liu X, Sayre J, Gilsanz V. Aortic calcification and the risk of osteoporosis and fractures. *J Clin Endocrinol Metab*. 2004;89(9):4246-53.
8. O'Neill TW, Felsenberg D, Varlow J, Cooper C, Kanis JA, Silman AJ. The prevalence of vertebral deformity in European men and women: the European Vertebral Osteoporosis Study. *J Bone Miner Res*. 1996;11(7):1010-8.
9. Naves M, Rodríguez-García M, Díaz-López JB, Gómez-Alonso C, Cannata-Andía JB. Progression of vascular calcifications is associated with greater bone loss and increased bone fractures. *Osteoporos Int*. 2008;19(8):1161-6.
10. Evrard S, Delanaye P, Kamel S, Cristol JP, Cavalier E. Vascular calcification: from pathophysiology to biomarkers. *Clin Chim Acta*. 2015;438:401-14.
11. van de Loo PG, Soute BA, van Haarlem LJ, Vermeer C. The effect of Gla-containing proteins on the precipitation of insoluble salts. *Biochem Biophys Res Commun*. 1987;142(1):113-9.
12. Hunter GK, Hauschka PV, Poole AR, Rosenberg LC, Goldberg HA. Nucleation and inhibition of hydroxyapatite formation by mineralized tissue proteins. *Biochem J*. 1996;317(Pt 1):59-64.
13. Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, et al. Increased bone formation in osteocalcin-deficient mice. *Nature*. 1996;382(6590):448-52.
14. Tacey A, Qaradakhi T, Brennan-Speranza T, Hayes A, Zulli A, Levinger I. Potential role for osteocalcin in the development of atherosclerosis and blood vessel disease. *Nutrients*. 2018; 10(10):1426.
15. Levy RJ, Gundberg C, Scheinman R. The identification of the vitamin K-dependent bone protein osteocalcin as one of the gamma-carboxyglutamic acid containing proteins present in calcified atherosclerotic plaque and mineralized heart valves. *Atherosclerosis*. 1983;46(1):49-56.
16. Levy RJ, Zenker JA, Lian JB. Vitamin K-dependent calcium binding proteins in aortic valve calcification. *J Clin Invest*. 1980;65(2):563-6.
17. Fleet JC, Hock JM. Identification of osteocalcin mRNA in nonosteoid tissue of rats and humans by reverse transcription-polymerase chain reaction. *J Bone Miner Res*. 1994;9(10):1565-73.
18. Johnson RC, Leopold JA, Loscalzo J. Vascular calcification: pathobiological mechanisms and clinical implications. *Circ Res*. 2006;99(10):1044-59.
19. Rashdan NA, Sim AM, Cui L, Phadwal K, Roberts FL, Carter R, et al. Osteocalcin Regulates Arterial Calcification Via Altered Wnt Signaling and Glucose Metabolism. *J Bone Miner Res*. 2020;35(2):357-67.
20. Millar SA, Patel H, Anderson SI, England TJ, O'Sullivan SE. Osteocalcin, Vascular calcification, and atherosclerosis: a systematic review and meta-analysis. *Front Endocrinol (Lausanne)*. 2017;8:183.
21. Kim KJ, Kim KM, Park KH, Choi HS, Rhee Y, Lee YH, et al. Aortic calcification and bone metabolism: the relationship between aortic calcification, BMD, vertebral fracture, 25-hydroxyvitamin D, and osteocalcin. *Calcif Tissue Int*. 2012;91(6):370-8.
22. Ogawa-Furuya N, Yamaguchi T, Yamamoto M, Kanazawa I, Sugimoto T. Serum osteocalcin levels are inversely associated with abdominal aortic calcification in men with type 2 diabetes mellitus. *Osteoporos Int*. 2013;24(8):2223-30.
23. Kanazawa I, Yamaguchi T, Yamamoto M, Yamauchi M, Kurioka S, Yano S, et al. Serum osteocalcin level is associated with glucose metabolism and atherosclerosis parameters in type 2 diabetes mellitus. *J Clin Endocrinol Metab*. 2009;94(1):45-9.
24. Confavreux CB, Szulc P, Casey R, Boutrou S, Varennes A, Vilaythiou N, et al. Higher serum osteocalcin is associated with lower abdominal aortic calcification progression and longer 10-year survival in elderly men of the MINOS cohort. *J Clin Endocrinol Metab*. 2013;98(3):1084-92.
25. Fusaro M, Gallieni M, Aghi A, Rizzo MA, Iervasi G, Nickolas TL, et al. Osteocalcin (bone GLA protein) levels, vascular calcifications, vertebral fractures and mortality in hemodialysis patients with diabetes mellitus. *J Nephrol*. 2019; 32(4):635-43.
26. Frazão JM, Martins P. Adynamic bone disease: clinical and therapeutic implications. *Curr Opin Nephrol Hypertens*. 2009;18(4):303-7.
27. Bover J, Ureña P, Brandenburg V, Goldsmith D, Ruiz C, DaSilva I, et al. Adynamic bone disease: from bone to vessels in chronic kidney disease. *Semin Nephrol*. 2014;34(6):626-40.
28. Ueda M, Inaba M, Okuno S, Maeno Y, Ishimura E, Yamakawa T, et al. Serum BAP as the clinically useful marker for predicting BMD reduction in diabetic hemodialysis patients with low PTH. *Life Sci*. 2005;77(10):1130-9.
29. O'Neill TW, Marsden D, Silman AJ. Differences in the characteristics of responders and non-responders in a prevalence survey of vertebral osteoporosis. *European Vertebral Osteoporosis Study Group. Osteoporos Int*. 1995;5(5):327-34.
30. Naves M, Díaz López JB, Virgós MJ, O'Neill TW, Gómez C, Zaplana J, et al. Índices de participación y aspectos metodológicos de interés en un estudio de prevalencia de fractura vertebral en Asturias. *REEMO*. 1993;2(5): 29-32.