Implication of osteoprotegerin and sclerostin in axial spondyloarthritis cardiovascular disease: a study of 163 Spanish patients

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Abstract

Objective

Due to the high incidence of cardiovascular disease in axial spondyloarthritis (axSpA), the search of potential biomarkers that may help to identify patients with high cardiovascular risk is of main importance. Therefore, in this study we assessed the implication of osteoprotegerin (OPG) and sclerostin (SCL), two biomarkers associated with cardiovascular disease and bone metabolism, in the clinical spectrum and atherosclerotic disease of patients with axSpA.

Methods

OPG and SCL serum levels were determined in 163 axSpA Spanish patients (119 ankylosing spondylitis and 44 non-radiographic axSpA) and 63 healthy controls by enzyme-linked immunosorbent assay. Carotid ultrasound was performed in axSpA patients to determine the presence of subclinical atherosclerosis (by the identification of abnormally increased carotid intima-media thickness [cIMT] and presence of plaques).

Results

Patients displayed higher OPG but lower SCL levels than controls (p=0.02 and 0.001, respectively). Association of these molecules with some metabolic syndrome features was seen. In this regard, OPG negatively correlated with body mass index (p=0.04) whereas SCL levels were higher in hypertensive patients (p=0.01) and in men (p=0.002). However, serum OPG and SCL were not significantly correlated with cIMT values or presence of plaques when data were adjusted by age at the time of the study, sex, classic cardiovascular risk factors and anti-TNF therapy.

Conclusion

Our results suggest an association of OPG and SCL in axSpA with some metabolic syndrome features that are associated with an increased risk of CV disease.

Key words

axSpA, osteoprotegerin, sclerostin, atherosclerosis, biomarkers

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Received on May 2, 2017; accepted in revised form on August 1, 2017.

Funding: F. Genre is a recipient of a Sara Borrell post-doctoral fellowship from the Instituto de Salud Carlos III (ISCIII), at the Spanish Ministry of Health (Spain) (CD15/00095).
S. Remuzgo-Martínez and B. Ubilla are supported by funds from the RETICS Programme (RIER) from the ISCIII, Spain (RD16/0012/0009 and RD12/0009/0013, respectively).
R. López-Mejías is supported by funds of the Miguel Servet type I programme from the ISCIII, Spain (CP16/00033).
This research was partly funded by a grant of the Sociedad Cántabra de Reumatología (Spain) awarded to J. Rueda-Gotor.

Competing interests: none declared.

Introduction

Axial spondyloarthritis (axSpA) is a chronic, inflammatory disease that principally affects the spine and pelvic joints (1). axSpA prevails in people under 45 years old and is characterised by chronic low back pain and stiffness of the spine, leading in some cases to spinal deformity and dysfunction that can be observed on plain radiographs in patients with ankylosing spondylitis (AS) (2), the prototype of axSpA. Other patients included under the term nonradiographic axSpA (nr-axSpA) do not show radiographic sacroiliitis, but display other features of axSpA (3). Whether AS and nr-axSpA are either two stages of the same disease or different diseases is still a contentious issue (1, 3-5). While it is true that some nr-axSpA patients evolve into AS over time, a proportion of nr-axSpA patients do not progress, not developing radiographic sacroiliitis (1). Besides, AS patients undergo a more severe disease progression in terms of inflammation, also characterised by longer disease duration when compared to nr-axSpA patients. Moreover, while AS is a male-dominant disease, nr-axSpA is more frequent in females (5).

The main symptoms of xSpA can also be accompanied by extra-articular manifestations, such as uveitis, inflammatory bowel disease (IBD) or psoriasis (1, 6, 7). Additionally, a body of evidence demonstrates that axSpA patients have a higher prevalence of cardiovascular (CV) risk factors grouped under the term metabolic syndrome (obesity, hypertension, insulin resistance and dyslipidaemia), promoting subsequently a higher risk of developing atherosclerosis and CV disease (8-14). The increased incidence of CV disease, along with the structural irreversible damage of the axial skeleton and subsequent reduction in the quality of life, lead to a worse prognosis of patients diagnosed with axSpA. However, the studies performed so far on CV disease in axSpA are mainly focused on AS. For this reason, in previous studies our group evaluated the incidence of atherosclerotic disease in axSpA (including both AS and nr-axSpA patients), by determining the presence of plaques or abnormal carotid intima-media thickness (cIMT) values (as surrogate markers of CV disease) by carotid ultrasonography studies (15, 16). The use of this imaging technique has been proven useful to assess CV risk in patients with rheumatic diseases such as AS (17). It is known that the presence of carotid plaques reflect an advanced stage of atherosclerosis, indicating thus high CV risk (18). Abnormal cIMT values are indicators of vascular wall hypertrophy, instead (19, 20). In this respect, we reported that carotid plaques were more frequent in axSpA patients than controls (15), in accordance with previous results on patients with AS (10). No significant differences were observed regarding cIMT values between axSpA patients and healthy controls in that study (15).

The search of potential biomarkers that may help to identify axSpA patients with high CV risk is of main importance. Since axSpA (particularly AS) is characterised by changes in the osteoproliferative process (7, 21), a dysregulation in the molecules involved in bone remodeling is highly plausible, also affecting vascular calcification in the context of atherosclerotic disease. Accordingly, in the present work we studied osteoprotegerin (OPG) and sclerostin (SCL), two biomarkers linked to bone metabolism and CV disease. OPG is a member of the tumour necrosis factor (TNF) receptor super-family that inhibits osteoclastogenesis and bone resorption by decreasing the binding of the receptor activator of nuclear factor-κB (RANK) to its ligand, RANKL (22). In addition, OPG neutralises TNF-related apoptosis inducing ligand (TRAIL), a molecule with anti-inflammatory and anti-atherosclerotic functions (23). Furthermore, it has been reported that OPG upregulates the production of endothelial adhesion molecules and enhances leukocyte adhesion to the endothelium, important steps in the onset of the atherosclerotic process (24, 25). Accordingly, and considering that OPG has been related to increased risk of atherosclerosis and CV disease in the general population (26), OPG was proposed as a potential biomarker of atherosclerosis (27, 28). Nevertheless, there is only a single report that evalu-

ated the role of OPG on atherosclerotic disease in a small cohort of AS patients (29). Regarding SCL, this is a glycoprotein that antagonises the Wnt/β-catenin canonical pathway, being thus a key modulator of bone metabolism. By binding to its co-receptors, SCL inhibits osteoblastogenesis and bone formation. Moreover, SCL was linked to vascular calcification, a commonly observed phenomenon in atherosclerotic disease (30). Similarly to OPG, high levels of circulating SCL have been associated with increased risk of CV events (31, 32). However, to the best of our knowledge, there are no previous studies on the implication of SCL in the atherosclerotic process in axSpA patients.

Therefore, the potential key role of OPG and SCL in atherosclerosis and the paucity of studies in this regard in axSpA prompted us to assess the implication of these molecules in the clinical spectrum of the disease and its association with features linked to the atherosclerotic process and CV disease in a large cohort of Spanish axSpA patients (including both AS and nr-axSpA, representing 73 and 27% of the patients, respectively).

Materials and methods

Patients and controls

For experiments involving humans and human blood samples, methods were carried out in accordance with the approved guidelines and regulations, according to the Declaration of Helsinki. All experimental protocols were approved by the Ethics Committee of Clinical Research of Cantabria (CEIC-C, reference number 7/2016). Informed consent was obtained from all subjects. 207 consecutive Spanish patients diagnosed with axSpA seen over a 3 year period at Hospital Universitario Marqués de Valdecilla and Hospital de Laredo (Cantabria, Spain) that fulfilled the ASAS classification criteria (3) were recruited for this study. Patients who had experienced CV events (n=5), patients with diabetes mellitus (n=8), chronic kidney disease (n=2), IBD (n=13) or psoriasis (n=16) were excluded from the study to avoid potential bias in our results. Consequently, 163 axSpA patients were finally included in the study. Of them, 44 fulfilled the definitions for nr-axSpA (3), while 119 also fulfilled definitions for AS according to the 1984 modified New York criteria (2). For the comparative analysis with axSpA patients regarding serum levels of OPG and SCL, 63 controls were recruited in primary health centres of Cantabria, who did not have history of CV events, chronic kidney disease, diabetes mellitus or chronic inflammatory diseases.

Clinical disease parameters such as Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Ankylosing Spondylitis Disease Activity Score (ASDAS), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Metrology Index (BASMI) and Maastricht Ankylosing Spondylitis Enthesitis Score (MASES) were evaluated in patients at the time of study. In addition, information on features of axSpA disease (history of hip involvement, synovitis, enthesitis, anterior uveitis, HLA-B27 status and disease duration) was also assessed. Data on body mass index (BMI), blood pressure, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglycerides at the time of study, and history of traditional CV risk factors (smoking, obesity, dyslipidaemia and hypertension) were collected. Obesity was defined if BMI (calculated as weight in kilograms divided by height in squared meters) was >30. Patients were considered to have dyslipidaemia if they had hypercholesterolaemia and/or hypertriglyceridaemia (defined as diagnosis of hypercholesterolaemia or hypertriglyceridaemia by the patients' family physician, or total cholesterol and/or triglyceride levels in fasting plasma being >220 and >150 mg/dL, respectively). In those patients with total cholesterol between 200 and 220 mg/dL, a diagnosis of dyslipidaemia was considered if the atherogenic index (total cholesterol/HDL-cholesterol) was ≥4.1. Patients were diagnosed as having hypertension if blood pressure was >140/90 mmHg or if they were taking antihypertensive agents. Information on C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) at the time of recruitment and at disease diagnosis was assessed. Information on therapy (including treatment with anti-TNF-α agents) from disease diagnosis was reviewed.

Assessment of surrogate markers of CV disease

All the axSpA patients underwent a carotid ultrasound study to assess the presence of abnormal cIMT values in the common carotid artery and presence of focal plaques in the extracranial carotid tree, as previously reported (15).

Study protocol

Determinations were made in the fasting state. Blood samples were taken for measurement of ESR (Westergren), CRP (latex immunoturbidimetry) and lipids (enzymatic colorimetry). In axSpA patients and healthy controls, OPG serum levels were determined by ELISA, as previously described (33). Commercial ELISA kits were used to measure serum SCL (TE1023HS, TECOmedical Group, San Diego, USA), according to the manufacturer's instructions. All samples were analysed in duplicate.

Statistical analysis

The data were first analysed in healthy controls and in the whole cohort of axSpA patients. Patients were later stratified into the two subtypes of axSpA: AS and nr-axSpA. The data were expressed as mean ± standard deviation (SD) for continuous variables, and number of individuals (n) and percentage (%) for categorical variables. Differences in OPG and SCL levels among the study groups were assessed by ANOVA. Correlation between OPG and SCL with selected continuous variables was performed via estimation of the Pearson partial correlation coefficient (r). Associations between categorical clinical features and OPG and SCL concentrations were assessed by Student's t-test. The association of these molecules with plaques was tested by ANOVA. Adjustment was performed for potential confounding factors: age at the time of the study, sex, classic CV risk factors (smoking, obesity, dyslipidaemia and hypertension) and anti-TNF-α treatment.

Two-sided p values <0.05 were considered to indicate statistical significance.

Table I. Demographical, clinical and laboratory data in healthy controls and patients with axSpA (both AS and nr-axSpA patients).

Variable	Controls (n=63)	axSpA (n=163)	AS (n=119)	nr-axSpA (n=44)
Men/Women, n	28/35	90/73	73/46	17/27
Age at study (years), mean ± SD	50.9 ± 15.3	43.7 ± 11.6	44.9 ± 11.9	40.3 ± 10.3
Age at axSpA diagnosis (years), mean ± SD	-	37.2 ± 10.4	36.7 ± 10.7	38.7 ± 9.2
Disease duration since axSpA diagnosis (years), mean ± SD	-	6.5 ± 8.8	8.3 ± 9.6	1.6 ± 2.8
History of classic cardiovascular risk factors, n (%)				
Current smokers	12 (19.0)	47 (28.8)	39 (32.8)	8 (18.2)
Obesity	12 (19.0)	32 (19.6)	25 (21.0)	7 (15.9)
Dyslipidaemia	13 (20.6)	33 (20.2)	25 (21.0)	8 (18.2)
Hypertension	12 (19.0)	25 (15.3)	19 (16.0)	6 (13.6)
Body mass index (kg/m^2) at study, mean ± SD	26.8 ± 4.9	25.9 ± 4.5	26.1 ± 4.6	25.2 ± 4.2
Systolic blood pressure (mm Hg) at study, mean ± SD	127.4 ± 15.4	128.1 ± 15.2	129.2 ± 15.0	125.2 ± 15.5
Diastolic blood pressure (mm Hg) at study, mean ± SD	78.2 ± 8.8	77.9 ± 10.0	78.0 ± 10.3	77.8 ± 9.2
Total cholesterol (mg/dL) at study, mean ± SD	202.2 ± 33.9	193.1 ± 35.6	194.9 ± 36.1	188.0 ± 34.1
HDL cholesterol (mg/dL) at study, mean ± SD	59.1 ± 16.0	55.4 ± 16.4	53.8 ± 14.8	59.5 ± 19.6
LDL cholesterol (mg/dL) at study, mean ± SD	122.0 ± 31.8	118.2 ± 30.0	120.7 ± 31.1	111.4 ± 25.8
Triglycerides (mg/dL) at study, mean ± SD	94.7 ± 47.7	97.5 ± 54.5	98.7 ± 54.4	94.3 ± 55.3
Atherogenic index (total cholesterol/HDL), mean ± SD	3.6 ± 1.1	3.7 ± 1.0	3.8 ± 1.0	3.4 ± 0.9
BASDAI at study, mean ± SD	-	3.8 ± 2.2	3.6 ± 2.2	4.4 ± 2.3
ASDAS at study, mean ± SD	-	2.4 ± 1.0	2.3 ± 1.0	2.4 ± 1.0
BASFI at study, mean ± SD	-	3.8 ± 2.5	3.8 ± 2.5	3.8 ± 2.4
BASMI at study, mean ± SD	-	2.8 ± 1.7	3.0 ± 1.7	2.2 ± 1.5
MASES at study, mean ± SD	-	2.1 ± 2.5	2.1 ± 2.3	2.4 ± 2.8
Extra-articular manifestations, n (%)	-	30 (18.4)	24 (20.2)	6 (13.6)
History of synovitis or enthesitis, n (%)	-	86 (52.8)	63 (52.9)	23 (52.3)
History of hip involvement, n (%)	-	10 (6.1)	10 (8.4)	0 (0.0)
Syndesmophytes at study, n (%)	-	51 (31.3)	48 (40.3)	3 (6.8)
HLA-B27 positive, n (%)	-	112 (68.7)	92 (77.3)	20 (45.5)
Anti-TNF-α therapy, n (%)	-	49 (30.1)	43 (36.1)	6 (13.6)
CRP (mg/L) at study, mean ± SD	2.9 ± 4.4	5.7 ± 9.9	6.3 ± 10.9	4.0 ± 5.8
CRP (mg/L) at axSpA diagnosis, mean ± SD	-	11.5 ± 23.4	14.0 ± 26.6	4.9 ± 7.4
ESR (mm/1st hour) at study, mean ± SD	11.5 ± 4.4	11.3 ± 12.4	12.3 ± 13.3	8.4 ± 8.9
ESR (mm/1st hour) at axSpA diagnosis, mean± SD	-	15.1 ± 17.9	17.2 ± 20.2	10.3 ± 10.2
Carotid IMT (mm), mean ± SD	-	0.608 ± 0.131	0.622 ± 0.14	0.568 ± 0.11
Carotid plaques, n (%)	-	51 (31.3)	43 (36.1)	8 (18.2)
OPG (ng/mL ± SD)	3.99 ± 1.66	4.52 ± 1.94	4.60 ± 1.97	4.31 ± 1.85
SCL (ng/mL ± SD)	0.43 ± 0.17	0.34 ± 0.11	0.35 ± 0.11	0.32 ± 0.11

AS: ankylosing spondylitis; ASDAS: Ankylosing Spondylitis Disease Activity Score; axSpA: axial spondyloarthritis; BASDAI: Bath Ankylosing Spondylitis Disease Activity Index; BASFI: Bath Ankylosing Spondylitis Functional Index; BASMI: Bath Ankylosing Spondylitis Metrology Index; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; HDL: high-density lipoprotein; HLA: human leukocyte antigen; IMT: intima-media thickness; LDL: low-Density Lipoprotein; MASES: Maastricht Ankylosing Spondylitis Enthesitis Score; nr-axSpA: non-radiographic axial spondyloarthritis; OPG: osteoprotegerin; SCL: sclerostin; SD: standard deviation; TNF: tumour necrosis factor.

Statistical analysis was performed using STATA® v. 11.1 (StataCorp, College Station, TX, USA).

Results

Baseline characteristics of the study population

The main demographic, clinical and laboratory data of controls and patients are shown in Table I. Regarding axSpA patients, 90 (55.2%) were men, 10 (6.1%) had hip involvement, and 86 (52.8%) had synovitis and/or enthesitis in other peripheral joints. Also, 30 (18.4%) had experienced extra-articular manifestations, 51 (31.3%) had syndesmophytes on plain radiographs, 112 (68.7%) were HLA-B27 positive, and 51 (31.3%) displayed carotid plaques. The mean age of patients ± SD was 43.7±11.6 years. The mean disease duration at the time of the study ± SD was 6.5±8.8 years. The mean BASDAI and ASDAS value ± SD was 3.8±2.2 and 2.4±1.0, respectively.

OPG and SCL levels in axSpA patients and controls

OPG levels were higher in axSpA patients than in controls (4.52±1.94 *vs*. 3.99±1.66 ng/mL, respectively, p=0.02, Table I, Fig. 1A). In contrast, axSpA patients displayed lower SCL levels than controls (0.34±0.11 *vs*. 0.43±0.17 ng/mL, respectively, p=0.001, Table I, Figure 1B). No statistically significant differences were observed in OPG or SCL levels between AS and nr-axSpA (p>0.05).

Relationship of OPG and SCL levels with main clinical features and routine laboratory parameters

Regarding OPG, a negative correlation emerged with BMI in axSpA (r=-0.166, p=0.04), which was further confirmed in the subgroup of AS patients (r=-0.192, p=0.04). No statistically significant association was observed in this regard in controls (p=0.65).

As for SCL, we found that SCL levels were higher in men than in women (0.37±0.12 *vs*. 0.31±0.10 ng/mL, p=0.002). This difference was also disclosed in AS and nr-axSpA (0.37±0.12 *vs*. 0.33±0.10 ng/mL, respectively, p=0.06 in AS; 0.37±0.12 *vs*. 0.29±0.10 ng/mL, respectively, p=0.02 in nr-

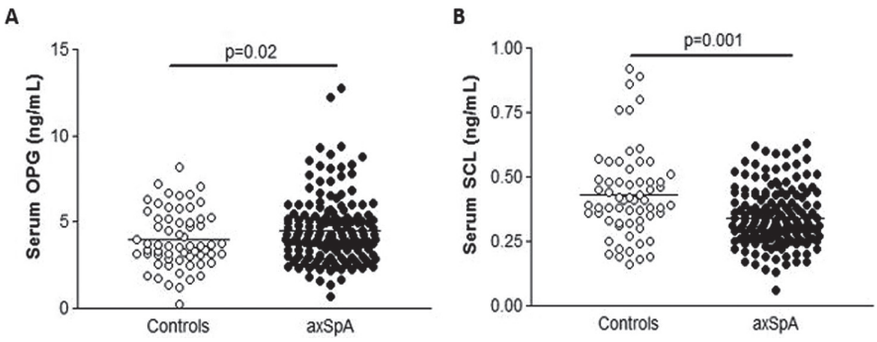

Fig. 1. Differences in OPG (A) and SCL (B) serum levels between healthy controls and axSpA patients, after adjustment for age at study, sex and traditional CV risk factors (smoking, obesity, dyslipidaemia and hypertension).
Healthy controls (n=63) are represented by empty circles (∘), while axSpA patients (n=163) are represented by filled circles (•). Horizontal bars indicate mean value of each group.

Table II. Univariate association between type of carotid plaques and SCL levels in patients with axSpA (both AS and nr-axSpA patients).

	SCL								
	axSpA			AS			nr-axSpA		
Carotid plaques	Mean ± SD (ng/mL)	*p*	*p**	Mean ± SD (ng/mL)	*p*	*p**	Mean ± SD (ng/mL)	*p*	*p**
No plaques	0.32 ± 0.10			0.33 ± 0.11			0.29 ± 0.10		
Unilateral plaques	0.36 ± 0.11	**0.0001**	0.28	0.35 ± 0.11	**0.007**	0.53	0.42 ± 0.13	**0.002**	0.11
Bilateral plaques	0.42 ± 0.11			0.42 ± 0.11			0.46 ± 0.09		

AS: ankylosing spondylitis; axSpA: axial apondyloarthritis; nr-axSpA: non-radiographic axial spondyloarthritis; SCL: sclerostin; SD: standard deviation.
Significant results are highlighted in bold ($p<0.05$). *p*-values for the comparison between the 3 groups obtained by one-way ANOVA are shown. *p** reflect *p*-values obtained by ANOVA after adjustment for age at the time of the study, sex, classic cardiovascular risk factors and anti-TNF-α treatment.

axSpA), although in AS it was marginally significant. Additionally, we observed that hypertensive axSpA patients had higher SCL levels compared to normotensive patients (0.40±0.13 *vs*. 0.33±0.11 ng/mL, respectively, $p=0.01$). After stratification into AS and nr-axSpA, AS patients with hypertension showed higher levels of SCL than those with normal blood pressure (0.41±0.13 *vs*. 0.34±0.10 ng/mL, respectively, $p=0.02$). No significant results were obtained when these associations were tested in controls ($p=0.48$ for sex and $p=0.67$ for hypertension).
OPG and SCL did not show association with disease activity or with specific clinical features of the disease (including BASDAI, ASDAS, BASFI, BASMI, MASES, hip involvement, synovitis, enthesitis, anterior uveitis, HLA-B27 status, disease duration, CRP and ESR levels) (*p*-values >0.05 in all the cases).

Association of OPG and SCL levels with surrogate markers of CV disease
We noticed that axSpA patients with carotid plaques had higher SCL levels than patients without plaques (0.39±0.11 *vs*. 0.32±0.10, $p=0.0001$). It was also true when patients were assessed according to the categories of AS (0.38±0.11 *vs*. 0.33±0.11 ng/mL, $p=0.01$) and nr-axSpA (0.44±0.11 *vs*. 0.29±0.10 ng/mL, $p=0.0004$). We also observed that patients with bilateral plaques had the highest SCL levels, followed by patients with unilateral plaques, while those without plaques showed the lowest SCL levels ($p=0.0001$ in axSpA, $p=0.007$ in AS and $p=0.002$ in nr-axSpA, Table II). However, no statistical significance was disclosed in all these analyses after adjustment for potential confounding factors ($p>0.05$, Table II).
When we assessed the association of SCL with cIMT values in axSpA, no statistically significant association was found ($p>0.05$). Likewise, when we evaluated the potential association of OPG with presence of plaques or cIMT values in axSpA, no statistically significant results were obtained ($p>0.05$).

Discussion

CV disease is a major cause of morbidity and mortality in chronic inflammatory diseases, among them axSpA (12). Therefore, the increased CV risk in these patients constitutes a primary cause of concern among rheumatologists. Consequently, therapeutic strategies in these patients should be targeted not only to treat the main symptoms of axSpA, but also to avoid the development of comorbidities, such as CV disease. Hence, the identification of biomarkers that could be used to assess CV risk in axSpA may be useful for the early diagnosis and more personalised treatment of patients, leading to a better quality of life.
In this context, and taking into consideration the higher incidence of CV disease and alterations in bone metabolism of axSpA patients, we decided to study the role of OPG and SCL, two molecules linked to both processes, in a large cohort of axSpA patients (including both AS and nr-axSpA patients). Surprisingly, even if an important function of these molecules might be expected in the atherosclerotic process in this pathology, previous reports in this regard are scarce (29). In addition, regarding the different subtypes of axSpA, it has been described that AS patients have a higher incidence of carotid plaques than controls (10), while such difference was not observed in nr-axSpA patients (16). However, whether AS and nr-axSpA are different entities or two phases of the same disease is still a matter of debate. Even if many clinical features are shared between them, others make them diverge, such as disease duration, inflammatory burden, severity and gender ratios. For this reason, in this study we further aimed to assess whether our results were also confirmed in the two subtypes of axSpA.
In the first place, we found higher OPG levels in axSpA patients when compared to controls, probably reflecting an increased CV risk, as previously

shown in the general population and other pathologies (26, 33-39). Also, and in line with previous reports of studies performed in patients with rheumatoid arthritis (33), coronary artery disease (40), type 1 diabetes mellitus (41) and obese individuals (42), we disclosed an inverse correlation between OPG levels and BMI in axSpA. An increase in fat mass, and subsequently in the levels of adipokines mainly produced by adipose tissue (*e.g.* leptin), may lead to a reduction of OPG production, either in a direct or indirect fashion, as wisely suggested by Dimitri *et al*. (43). This finding may have a potential relevance because obesity is a well-known component of the metabolic syndrome that is frequently observed in patients with inflammatory rheumatic diseases (44). In contrast to the results reported by Ashley *et al*. in the general population (45), we did not observe any correlation between OPG and BMI in our healthy controls cohort. Further studies should be performed to determine whether this association is specific of inflammatory diseases or not, or if the link between OPG and BMI is more pronounced in inflammatory diseases.

Regarding markers of subclinical atherosclerosis, even though the majority of studies performed in other conditions different from axSpA and in the general population show a positive correlation of such markers with OPG (27, 46-48), a study on coronary artery disease did not show such an association between them (49). In keeping with that, in our study we did not find any association between OPG and presence of plaques or cIMT values. Our results support the data recently reported in a smaller cohort of AS patients, in which no association was found between OPG serum levels and cIMT values or arterial stiffness (29). Nevertheless, it is important to notice that, in a previous study of our group performed in AS patients undergoing anti-TNF-α therapy, OPG serum levels correlated with asymmetric dimethylarginine, a biomarker of endothelial cell activation (28). Similarly, another study also showed a positive association between OPG and angiopoietin-2, another molecule linked to endothelial cell activation (24). All these data suggest that probably OPG plays an indirect role on the atherosclerotic process, by interacting with other molecules implicated in this process. The fact that the presence of OPG was previously reported in atherosclerotic plaques further supports the relevant role of OPG in this affection (50-52).

Secondly, we assessed the implication of SCL in our cohort of axSpA patients. In this regard, we observed lower SCL levels in axSpA when compared to controls. This finding makes sense if we consider that SCL has an inhibitory effect on bone formation (30) and AS is mainly characterised by pathologic new bone formation. In fact, our results are in accordance with those previously obtained (21, 53, 54). Furthermore, and in keeping with previous studies (55, 56), men showed higher SCL levels than women in both types of axSpA. The higher SCL levels in men could be explained by an inhibitory effect of estrogen on this protein, as previously reported (57, 58). Additionally, in assessing metabolic syndrome features, we disclosed that hypertensive AS patients had higher SCL levels. This also seems reasonable considering that vascular calcification, which frequently occurs in atherosclerotic lesions, leads to arterial stiffness and triggers hypertension (59). In this line, SCL has recently been proposed as a predictor of arterial stiffness (60, 61). The fact that no difference was observed in SCL levels in controls when stratified according to hypertension suggests that the relationship between these variables may be specific of the disease. Previous studies on the association between SCL and cIMT performed in patients with type-2 diabetes mellitus and in the general population showed contradictory results (61-63). In this regard, we did not find any association of SCL with cIMT values. Notwithstanding, we observed that axSpA patients with carotid plaques displayed higher SCL levels than those without plaques. Furthermore, when patients were stratified according to the distribution of plaques, we found that SCL levels were higher in patients with bilateral plaques than those with unilateral plaques, and in both cases those levels were increased compared to patients who did not develop plaques. This is in line with previous studies that reported a positive association between SCL levels and aortic calcification and plaques (30, 63, 64). This supports the idea that SCL is upregulated in the vascular wall to counter-regulate vascular calcification (30). However, it is important to mention that the association between SCL levels and presence of plaques found in our study was lost after adjustment for potential confounding factors. This may be indicating the involvement of a third factor in the association between SCL and plaques. Further studies are warranted to elucidate the exact mechanisms by which SCL exerts its action in the atherosclerotic process.

In summary, the data obtained in our study suggest that, even if OPG and SCL were not significantly associated with surrogate markers of CV disease, they could be playing an indirect role in the development of CV disease in axSpA, possibly mediated by their influence on some metabolic syndrome features. Given that the number of nr-axSpA patients and healthy controls included in our series could be somehow limited and that statistical significance for the association between SCL levels and the presence of plaques was no longer significant after adjustment for potential confounding factors, further studies should be performed in this regard. It would be interesting to assess other aspects of OPG and SCL biology in axSpA and atherosclerosis to determine if these molecules are involved in the pathogenesis of atherosclerosis in axSpA, a mere by-product of the process or a result of an homeostatic adjustment to deal with the increased CV risk in these patients. This may shed light on the precise mechanisms for the role of OPG and SCL in this process. Therefore, the use of serum levels of OPG and SCL as predictors and diagnostic tools of CV disease in the clinical setting needs to be elucidated in future studies.

Acknowledgements

We wish to thank all the patients and controls that participated in this study. We would like to specially thank Jesús González Vela and Patricia Fuentevilla for their technical assistance.

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