Cardiovascular Diabetology

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Bone proteins are associated with cardiovascular risk according to the SCORE2-Diabetes algorithm

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Abstract

Background Typical bone proteins, such as sclerostin and periostin, have been associated with cardiovascular disease (CVD). Simultaneously, several risk scores have been developed to predict CVD in the general population. Therefore, we aimed to evaluate the association of these bone proteins related to CVD, with the main vascular risk scales: Framingham Risk Score (FRS), REGICOR and SCORE2-Diabetes, in patients with type 2 diabetes. We focus in particular on the SCORE2-Diabetes algorithm, which predicts 10-year CVD risk and is specific to the study population.

Methods This was a cross-sectional study including 104 patients with type 2 diabetes (62 ± 6 years, 60% males). Clinical data, biochemical measurements, and serum bioactive sclerostin and periostin levels were collected, and different risk scales were calculated. The association between bioactive sclerostin or periostin with the risk scales was analyzed.

Results A positive correlation was observed between circulating levels of bioactive sclerostin ($p < 0.001$) and periostin ($p < 0.001$) with SCORE2-Diabetes values. However, no correlation was found with FRS or REGICOR scales. Both serum bioactive sclerostin and periostin levels were significantly elevated in patients at high-very high risk of CVD (score ≥ 10%) than in the low-moderate risk group (score < 10%) ($p < 0.001$ for both). Moreover, analyzing these proteins to identify patients with type 2 diabetes at high-very high vascular risk using ROC curves, we observed significant AUC values for bioactive sclerostin (AUC = 0.696; $p = 0.001$), periostin (AUC = 0.749; $p < 0.001$), and the model combining both (AUC = 0.795; $p < 0.001$). For diagnosing high-very high vascular risk, serum bioactive sclerostin levels > 131 pmol/L showed 51.6% sensitivity and 78.6% specificity. Similarly, serum periostin levels > 1144 pmol/L had 64.5% sensitivity and 76.2% specificity.

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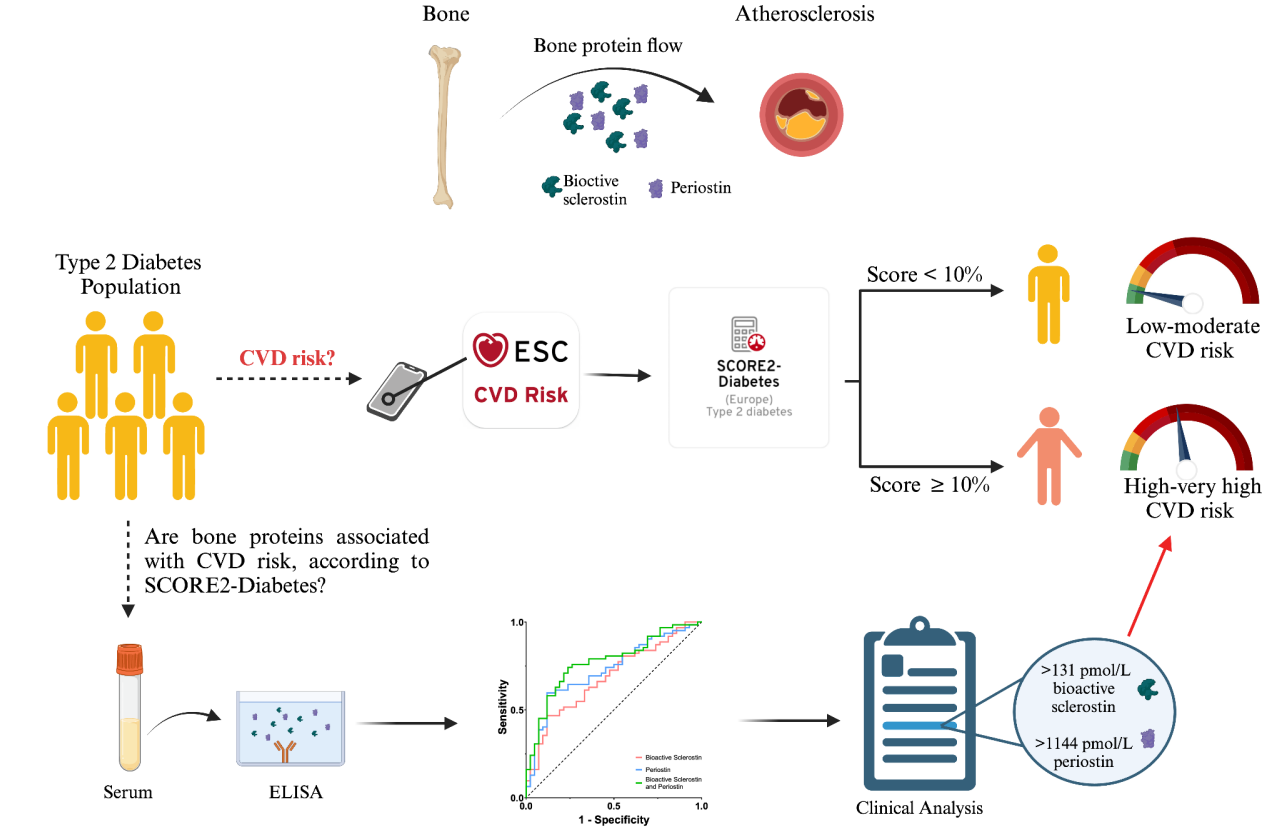
Conclusions Sclerostin and periostin are associated with vascular risk in the SCORE2-Diabetes algorithm, opening a new line of investigation to identify novel biomarkers of cardiovascular risk in the type 2 diabetes population.

Keywords Biomarker, Bone proteins, Cardiovascular disease risk, Periostin, Sclerostin, SCORE2-Diabetes, Type 2 diabetes

Graphical Abstract



CVD: Cardiovascular disease

Background

Type 2 diabetes is associated with an increased risk of cardiovascular disease (CVD) [1] affecting about 35% of these patients [2]. Therefore, cardiovascular risk assessment in patients with type 2 diabetes is a critical component in disease management. Several risk scores have been developed to predict CVD in the general population, such as the Framingham Risk Score (FRS) [3] and REGICOR [4], the latter being the result of validation of the Framingham equation in the Spanish population. SCORE2-Diabetes has recently been developed, an extension of the SCORE2 algorithms, specifically for patients with type 2 diabetes. This new algorithm has been developed, validated, and calibrated to predict 10-year CVD risk in people with type 2 diabetes by the European Society of Cardiology (ESC) [5]. Although these informatics tools are used in clinical practice, there is a need to explore new biomarkers that could be used for proper cardiovascular risk stratification in patients with type 2 diabetes.

The connection between bone metabolism and the vascular system has been suggested. In fact, bone proteins are found to play a crucial role not only in the maintenance of bone health, but also in cardiovascular disease, such as sclerostin and periostin. Sclerostin, a protein synthesized by the *SOST* gene, is an inhibitor of the Wnt/β-catenin pathway that regulates bone formation [6]. Under physiological condition, sclerostin is mainly expressed by osteocytes [7], although it is also expressed by vascular smooth muscle cells in calcifying environment [8]. In the serum level, sclerostin had been found highly expressed in patients with type 2 diabetes and atherosclerotic lesions [9, 10]. Also, several studies have shown an association between serum sclerostin levels and the occurrence of CVD and cardiovascular mortality [6, 11–13]. Recently, it has been shown that sclerostin could

play a protective role in the development of atherosclerosis in patients with type 2 diabetes [14]. These findings strongly support that, in addition to its role in regulating bone metabolism, sclerostin is also involved in vascular homeostasis and acts as an important modulator of the Wnt/β-catenin pathway in CVD. In addition, research suggests that sclerostin is involved in glucose and lipid metabolism, potentially influencing insulin resistance and obesity. In particular, studies have shown a strong association between sclerostin and insulin resistance in obese individuals, especially those with type 2 diabetes [15, 16].

Regarding periostin, is a ubiquitous protein originally known as osteoblast-specific factor-2 and belongs to a group of nonstructural extracellular matrix (ECM) proteins. This protein is expressed in the periosteum, is present in the ECM, and participates in cell-ECM interactions [17]. Its role in the ECM indicates that periostin plays a significant role in adverse cardiac remodeling. Studies have shown elevated expression of periostin in the hearts of diabetic rats, suggesting its involvement in the pathogenesis of diabetic cardiomyopathy [18, 19]. Furthermore, a strong relationship has been described between periostin overexpression and cardiac remodeling in human [20].

To date, the association between these bone proteins and cardiovascular risk assessment scores in patients with type 2 diabetes has not been studied. Therefore, this study aims to investigate whether serum levels of sclerostin and periostin are related to cardiovascular risk scales, in particular SCORE2-Diabetes.

Methods

Study population

This cross-sectional study included 104 patients with type 2 diabetes (age range 46–69, 60% male). Type 2 diabetes was diagnosed according to the American Diabetes Association criteria [21]. The recruitment of patients with type 2 diabetes was from 2017 to 2018 in the Endocrinology and Nutrition Unit of the University Hospital Clínico San Cecilio of Granada (Spain) according to the following criteria: Caucasian ethnicity and normal values for blood count. Exclusion criteria were as follows: hepatic diseases indicated by Aspartate Aminotransferase (AST) or Alanine Aminotransferase (ALT) levels > 3 × Upper Limit of Normal (ULN) or total bilirubin > 1.5 × ULN; gastrointestinal diseases such as inflammatory bowel disease (including Crohn's disease and ulcerative colitis) or malabsorption; active endocrinologic conditions such as hyperthyroidism, hypothyroidism, or Cushing's syndrome; and bone diseases including fragility fractures, osteomalacia, rickets, Paget's disease, osteogenesis imperfecta, and bone tumors. Additionally, patients with CVD, including myocardial infarction, coronary insufficiency, angina, ischemic stroke, transient ischemic attack, hemorrhagic stroke, peripheral artery disease, heart failure, or pacemaker implantation, were excluded. Participants with an estimated glomerular filtration rate (eGFR) below 45 mL/min/1.73 m^2 or those receiving treatment with thiazolidinediones, warfarin or other drugs that affect to bone metabolism, were also excluded. These rigorous criteria ensured the selection of suitable individuals for the study, minimizing potential confounding factors and enhancing the reliability of the research findings.

The Biobank of the Andalusian Public Health System at the University Hospital Clínico San Cecilio of Granada was responsible for the management of all samples used in this study. Prior to participation, informed consent was obtained from each patient ensuring their voluntary involvement. This study received ethical approval from the Granada Provincial Research Ethics Committee (Project ID: 0858-N-17) and it adhered to the principles outlined in the World Medical Association Declaration of Helsinki.

Clinical evaluation and biochemical measurements of study population

Height, weight, and waist circumference measurements were obtained according to standard procedures. The body mass index (BMI) was calculated by the Quetelet formula: weight (kg)/stature (m^2). Systolic and diastolic blood pressure was measured using a standard electronic sphygmomanometer (standard cuff 12–13 cm wide and 35 cm long). Patients rested for 5 min before the first measurement, and two readings were taken 1–2 minutes apart. If the initial readings differed by more than 10 mmHg, additional measurements were conducted. The final blood pressure was calculated as the average of the last two readings. Hypertension was defined as values equal to or exceeding 140/90 mmHg and/or the use of antihypertensive treatment. Dyslipidemia was characterized by serum levels of high-density lipoprotein cholesterol (HDL-c) < 50 mg/dL, low-density lipoprotein cholesterol (LDL-c) > 100 mg/dL, triglycerides (TG) > 150 mg/dL, and/or current medication with lipid-lowering drugs. Smoking, alcohol consumption, and physical activity levels were assessed using the Spanish version of the questionnaire for Rapid Assessment of Physical Activity [22]. For biochemical measurements, venous blood samples were collected in the morning after fasting overnight, and serum samples were stored at -80 °C until analysis. The parameters as fasting plasma glucose (FPG), glycated haemoglobin (HbA1c), lipids (total cholesterol, HDL-c, LDL-c, TG) and serum creatinine were measured using standard automated laboratory techniques. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation

(CKD-EPI) [23]. Serum undercarboxylated osteocalcin (ucOC) levels were measured in duplicate by enzyme-linked immunosorbent assay (ELISA) according to Takara Bio (MK118, Japan) instructions. Intra-assay and inter-assay variations precision testing were consistent with reported by the manufacturer (6–10% and 5.21–8.33%, respectively). Bioactive sclerostin and periostin levels were determined in duplicate using ELISA method, following the manufacturer's protocols (BI-20472 and BI-20433, respectively; Biomedica Medizinprodukte GmbH, Vienna, Austria), with detection ranges of 0–320 pmol/L for bioactive sclerostin and 20–4,000 pmol/L for periostin. Precision testing showed intra-assay and inter-assay variations of ≤5% and ≤1% for bioactive sclerostin, and ≤6% and ≤3% for periostin. According to the manufacturer, the expected circulating levels of bioactive sclerostin and periostin in healthy controls had mean values of 70.8 pmol/L and 864 pmol/L, respectively.

Cardiovascular disease risk outcome assessment

The 10-year risk of CV events in all patients was estimated by calculating risk scores with three different algorithms: FRS, REGICOR and SCORE2-Diabetes. FRS was calculated using age (30–74 years), sex, diabetes, smoking, systolic and diastolic blood pressure, total cholesterol, HDL-c, LDL-c, and TG as input variables [3]. Patients were accordingly divided into low risk (score < 15%; $n=59$), moderate risk (15% ≤ score < 20%; $n=20$), high risk (20% ≤ score < 30%; $n=15$), and very high risk (score ≥ 30%; $n=10$). Regarding REGICOR, is the Spanish-calibrated adaptation of the Framingham score; it is valid and reliable for the population without previous CVD aged between 35 and 74 years. REGICOR was estimated measuring the variables age, sex, diabetes, smoking, systolic and diastolic blood pressure, total cholesterol, and HDL-c [4]. In this case, the patients were divided into two groups according to whether they were low risk (score ≤ 10%; $n=90$) or high risk (score > 10%; $n=14$). As for SCORE2-Diabetes is a recent risk prediction algorithm developed, calibrated, and validated to estimate 10-year CVD risk specifically in individuals with type 2 diabetes but no prior CVD, aged 40–69 years and considers the different risk levels in four risk regions of Europe by the SCORE2-Diabetes Working Group and the ESC Cardiovascular Risk Collaboration. SCORE2-Diabetes was calculated select the region of the patients, age, age at diabetes diagnosis, current smoking, systolic blood pressure, total cholesterol, HDL-c, HbA1c and eGFR [5]. Patients were accordingly divided into low-moderate risk (score < 10%; $n=42$) and high-very high risk (score ≥ 10%; $n=62$). A statistical power analysis considering the proportion of patients with type 2 diabetes at high-very high cardiovascular risk according to SCORE2-Diabetes in our study population (59.61%) was performed. The scientific literature corroborates this proportion describing a mean prevalence of 61% of high-very high cardiovascular risk in patients with type 2 diabetes in Spain [24]. Considering our sample comprises 62 patients with type 2 diabetes at high-very high cardiovascular risk and 42 patients with type 2 diabetes at low-moderate cardiovascular risk our statistical power is 0.83. Calculations were obtained using G*Power (v.3.1.9.4), using proportions for two independent groups (Fisher's exact test) and an α significance of 0.05.

Statistical analysis

Analyses were performed using SPSS version 28.0.1.0 software (IBM Corp.) and RStudio. A Kolmogorov-Smirnov test was used to test the normality of distribution of the continuous variables. Associations between continuous variables were described by Pearson´s correlation coefficients. Data were expressed as means ± standard deviation (SD) for variables normally distributed. The mean values between groups were compared using the unpaired Student´s t-test or Welch's t-test for continuous and normally distributed variables. The data for categorical variables were presented as percentages and the $\chi 2$ test was used to compare categorical variables between groups. A multiple lineal regression model was performed to determine the variables independently associated with bioactive sclerostin or periostin (dependent variable), including variables associated to SCORE2-Diabetes as independent variables. Data were expressed as B; 95% confidence interval (CI) (lower limit/upper limit). To identify bioactive sclerostin or periostin as an independent predictor of CVD risk according to SCORE2-Diabetes, a multiple logistic regression models were performed. Statistical significance was set at $p<0.05$ (two tailed) and $p<0.05$ for multiple linear and logistic regression analysis. The performance of serum bioactive sclerostin or/and periostin as estimators of CVD risk according to SCORE2-Diabetes were assessed using receiver operating characteristic curve (ROC). The area under the curve (AUC) indicates the probability to predict an event. The Youden index was calculated to assess the effectiveness of a diagnostic marker (bioactive sclerostin and periostin), allowing the selection of a threshold value or optimal cut-off point for the biomarker of interest.

Results

Correlations between bone proteins and risk scales of CV events in patients with type 2 diabetes

A positive correlation was observed between serum bioactive sclerostin levels and SCORE2-Diabetes ($r=0.53$; 95%CI [0.37–0.65]; $p<0.001$) (Fig. 1A). However, serum levels of bioactive sclerostin did not correlate with FRS ($p=0.280$) or REGICOR ($p=0.261$) risk scores. Similarly,

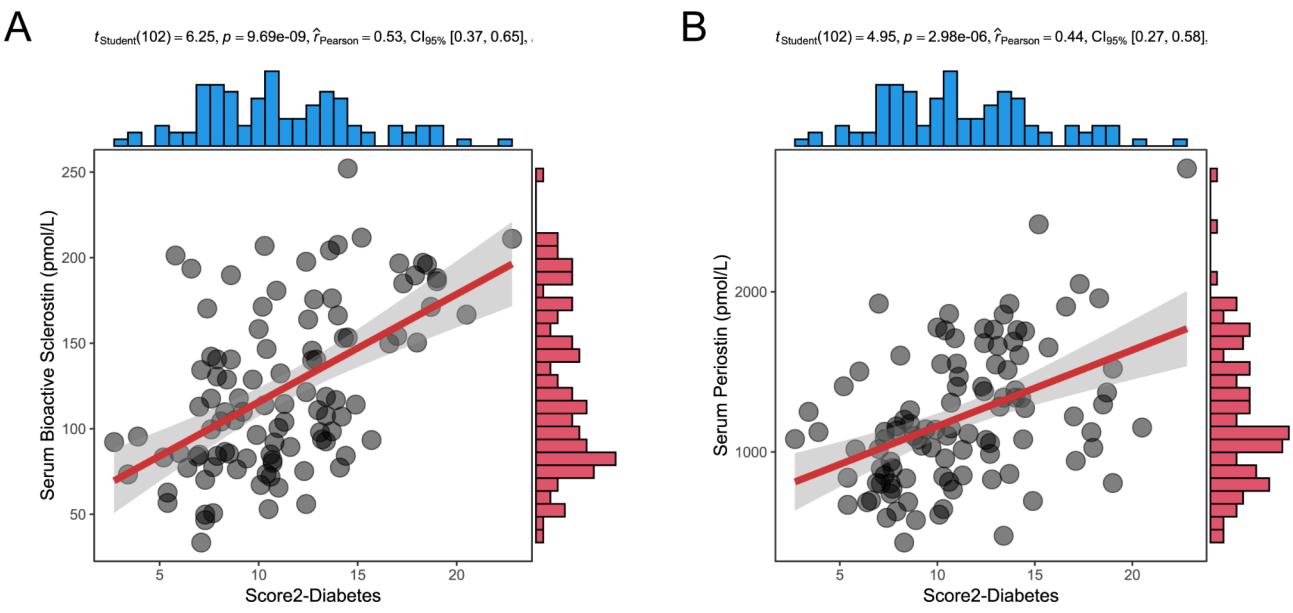


Fig. 1 Scatter plots showing the correlation between SCORE2-Diabetes and: **(A)** bioactive sclerostin (pmol/L), and **(B)** periostin (pmol/L), in type 2 diabetes patients ($n=104$). The *p*-values between the different associations were performed by Pearson´s correlation coefficients (showing $p<0.05$ in each scatter plot)

serum periostin levels showed a positive correlation with SCORE2-Diabetes ($r=0.44$; 95%CI [0.27–0.58]; $p<0.001$) (Fig. 1B). Nevertheless, serum levels of periostin did not correlate with FRS ($p=0.283$) and REGICOR ($p=0.319$) risk scores. These results demonstrated a relationship between both bone-typical proteins and the SCORE2-Diabetes algorithm.

Characteristics of the study population according SCORE2-Diabetes

Table 1 summarizes the clinical and biochemical parameters of the entire population, further comparing the groups divided according to SCORE2-Diabetes risk score: low-moderate risk (score<10%) and high-very high risk (score≥10%). Significant differences were observed between the groups with respect to sex, age, duration of diabetes, hypertension, and eGFR. Additionally, there were no significant differences between the groups in weight, height, BMI, or waist circumference.

Comparison of bioactive sclerostin & periostin values according to vascular risk levels in SCORE2-Diabetes

Serum bioactive sclerostin levels were notably higher in the group with a high-very high risk of CVD (score≥10%) compared to the low-moderate risk group (score<10%) (136±49 pmol/L vs. 104±40 pmol/L; $p<0.001$) (Fig. 2A). When segregated by sex, no significant differences in serum bioactive sclerostin levels were observed in the low-moderate risk group ($p=0.373$). However, in the high-very high risk group, males exhibited higher levels of bioactive sclerostin compared to females (151±47 pmol/L vs. 103±35 pmol/L; $p<0.001$). Regarding periostin, increased serum periostin levels were observed in the group with a high-very high risk of CVD compared to the low-moderate risk group (1355±447 pmol/L vs. 1012±317 pmol/L; $p<0.001$) (Fig. 2B). Furthermore, when categorized by sex, no differences in serum periostin levels were found in either the low-moderate risk group ($p=0.404$) or high-very high risk group ($p=0.134$).

Determinants of serum bioactive sclerostin & periostin levels in variables of SCORE2-Diabetes

To analyze the variables that influence the serum levels of bioactive sclerostin and periostin, a multiple linear regression analysis model was performed for each protein. The model included the SCORE2-Diabetes variables (age, age at diabetes diagnosis, current smoking, systolic blood pressure, total cholesterol, HDL-c, HbA1c and eGFR). The results of this analysis will provide valuable information on the multifactorial nature of bioactive sclerostin and periostin regulation and its associations with various factors included in the SCORE2-Diabetes algorithms. The results showed that the variables independently associated to the serum bioactive sclerostin level were age (B=0.211; 95% CI [0.236/3.331]; $p=0.024$), age at diabetes diagnosis (B =– 0.322; 95% CI [– 2.827/– 0.819]; $p<0.001$), systolic blood pressure (B=0.200; 95% CI [0.085/0.996]; $p=0.021$), and eGFR (B =– 0.383; 95% CI [– 1.582/– 0.593]; $p<0.001$). As for periostin, the results showed that the variables independently associated with the serum periostin level were age (B=0.270; 95% CI [5.528/35.618]; $p=0.008$), age

Table 1 Clinical and biochemical parameters of the whole population and intergroup comparison of patients with type 2 diabetes according to the SCORE2-Diabetes algorithm

	Patients with type 2 diabetes	SCORE2-Diabetes		
		Score < 10%	Score ≥ 10%	*p*
Patients *(n)*	104	42	62	0.217
Male/female (%)	60/40	48/52	68/32	0.040*
Age (years)	62 ± 6	58 ± 5	65 ± 5	< 0.001*
Body weight (kg)	89 ± 13	88 ± 14	89 ± 13	0.380
Height (cm)	166 ± 1	165 ± 8	166 ± 8	0.301
BMI (kg/m^2)	32 ± 4	32 ± 5	32 ± 4	0.451
Waist circumference (cm)	106 ± 8	106 ± 9	108 ± 11	0.220
Diabetes duration (years)	13 ± 9	9 ± 6	16 ± 9	< 0.001*
Dyslipidemia (%)	89	83	92	0.178
Hypertension (%)	88	76	90	0.050*
Systolic blood pressure (mmHg)	133 ± 18	127 ± 15	137 ± 18	0.001*
Diastolic blood pressure (mmHg)	80 ± 10	81 ± 9	80 ± 11	0.320
Smoker (%)	10	7	11	0.481
Alcohol consumption excessive (%)	15	7	21	0.055
Sedentarism (%)	16	6	23	0.031*
Insulin (%)	70	71	69	0.821
Oral antidiabetic drugs (%)	30	29	31	0.821
FPG (mg/dL)	149 ± 52	136 ± 34	158 ± 60	0.009*
HbA1c (%)	7.8 ± 1.4	7.5 ± 1	9 ± 1.5	0.029*
Total cholesterol (mg/dL)	167 ± 47	170 ± 53	164 ± 43	0.261
HDL-c (mg/dL)	44 ± 10	44 ± 10	44 ± 9	0.448
LDL-c (mg/dL)	94 ± 42	95 ± 46	93 ± 39	0.388
TG (mg/dL)	167 ± 78	167 ± 78	166 ± 79	0.473
eGFR (mL/min/1.73 m^2)	87 ± 17	93 ± 14	83 ± 18	0.001*
ucOC (ng/mL)	10 ± 5	10 ± 4	11 ± 6	0.264

BMI: body mass index; FPG: fasting plasma glucose; HbA1c: glycated haemoglobin; HDL-c: high-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; TG: triglycerides; eGFR: estimated glomerular filtration rate; ucOC: undercarboxylated osteocalcin. The data for continuous and normally distributed variables are presented as the mean ± SD. The data for categorical variables are presented as percentages. Student's t-test was used for comparisons of continuous and normally variables between groups. The χ2 test was used for the comparison of categorical variables between groups. *$p < 0.05$

at diabetes diagnosis (B =– 0.202; 95% CI [– 20.369/– 0.261]; $p = 0.044$), and systolic blood pressure (B = 0.207; 95% CI [0.471/9.607]; $p = 0.031$).

Impact of bioactive sclerostin & periostin levels on CVD risk in patients with type 2 diabetes according to SCORE2-Diabetes

Logistic regression models were employed to assess the association between serum bioactive sclerostin and periostin levels with SCORE2-Diabetes in patients with type 2 diabetes. Results indicated that serum bioactive sclerostin level (OR = 1.017; 95% CI [1.008/1.028]; $p < 0.001$) and serum periostin level (OR = 1.002; 95% CI [1.001/1.004]; $p < 0.001$) independently estimated CVD risk based on SCORE2-Diabetes. Therefore, in patients with type 2 diabetes, for each 10 pmol/L increase in serum bioactive sclerostin level, there is a 17% increased risk of CVD, and for each 100 pmol/L increase in serum periostin level, there is a 20% increased risk of CVD.

ROC curve analyses were conducted to assess the usefulness of serum levels of bioactive sclerostin and periostin in estimating cardiovascular risk based on SCORE2-Diabetes. Three models were tested: one with only bioactive sclerostin, another with only periostin, and the third with both proteins (full model). The AUC for bioactive sclerostin alone was 0.6965 (95%CI [0.583–0.787]; $p = 0.001$), for periostin alone it was 0.7499 (95%CI [0.634–0.829]; $p < 0.001$), and for the full model was 0.7952 (95%CI [0.683–0.864]; $p < 0.001$) (Fig. 3). According to this results, bioactive sclerostin values above 131 pmol/L presented a sensitivity of 51.6% and a specificity of 78.6% for the diagnosis of high-very high vascular risk, while periostin values above 1144 pmol/L presented a sensitivity of 64.5% and a specificity of 76.2% for the same diagnosis.

Discussion

Our study examines for the first time whether serum levels of sclerostin and periostin are related to cardiovascular risk scales, mainly SCORE2-Diabetes. Firstly, this study showed a positive correlation between serum levels of bioactive sclerostin and periostin with SCORE2-Diabetes. However, there were no correlation with FRS and REGICOR scales. Therefore, we focused on SCORE2-Diabetes for our study population. Secondly, increased level of circulating bioactive sclerostin was observed in the group of patients with type 2 diabetes at high-very high risk of CVD according to SCORE2-Diabetes (mainly in males). A similar increase in serum periostin levels was observed in the group of patients with type 2 diabetes at high-very high risk of CVD, although there was no gender difference. Finally, we provide both bioactive sclerostin and periostin values to diagnose patients with type 2 diabetes and high-very high vascular risk using ROC curves.

SCORE2-Diabetes is a recent risk prediction algorithm developed, calibrated, and validated to estimate 10-year CVD risk specifically in individuals with type 2 diabetes but no prior CVD, aged 40–69 years and considers the different risk levels in four risk regions of Europe. SCORE2-Diabetes including novel variables such as age at diabetes diagnosis, HbA1c and eGFR [5]. This scale emphasizes the importance of considering these

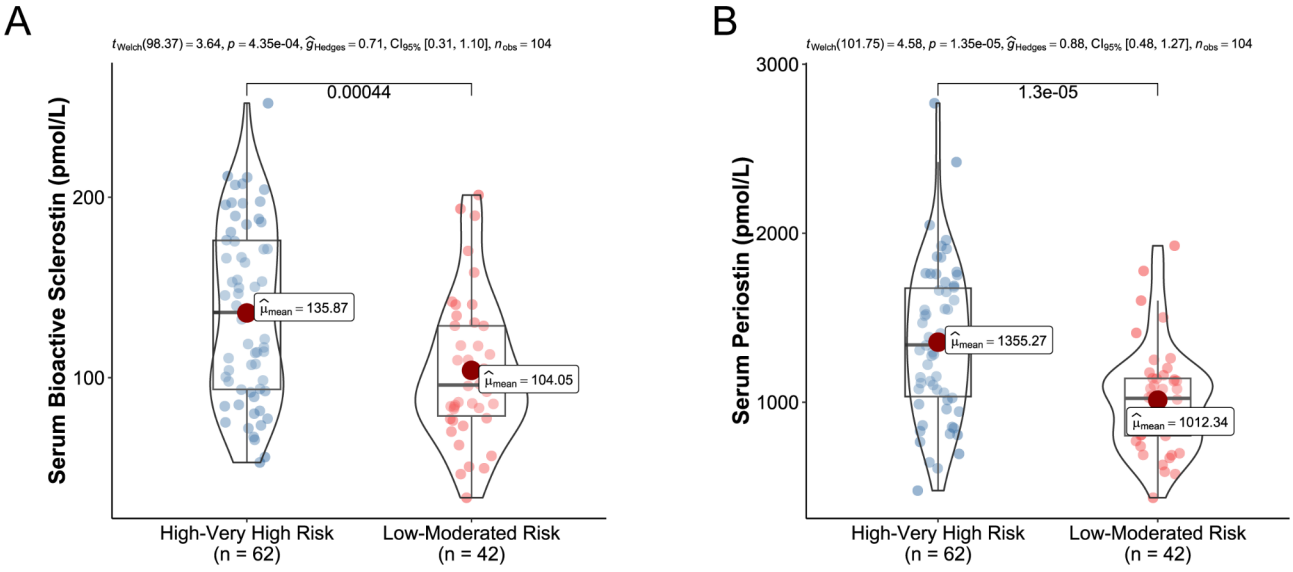


Fig. 2 Serum bioactive sclerostin and periostin levels in patients with type 2 diabetes according to SCORE2-Diabetes. **(A)** Serum bioactive sclerostin levels in the group with low-moderate risk of CVD (score < 10%; *n* = 42) and high-very high CVD risk group (score ≥ 10%; *n* = 62). **(B)** Serum periostin levels in the group with low-moderate risk of CVD (score < 10%; *n* = 42) and high-very high CVD risk group (score ≥ 10%; *n* = 62). The *p*-values between groups were calculated using Welch's t-test. Data are displayed as mean ± SD

additional diabetic variables to assess vascular risk in patients with type 2 diabetes, in contrast to the FRS and REGICOR scales. According to these diabetic variables including in SCORE2-Diabetes scale, our results have shown a significant difference in eGFR between the group of patients with type 2 diabetes at high-very high risk of CVD and those at low-moderate risk group. This finding aligns with previous studies that have closely related cardiovascular and renal diseases in patients with type 2 diabetes [25]. Furthermore, our results showed that eGFR was independently associated with the serum level of bioactive sclerostin, although there was no association with periostin. Consistent with these results, several studies have reported increased serum sclerostin levels in chronic kidney disease (CKD) patients with atherosclerotic CVD [26–28] and all-cause cardiovascular mortality in CKD patients [11, 29]. Therefore, the elevated bioactive sclerostin levels observed in the high-very high cardiovascular risk group could partly be attributed to mildly impaired renal function, as opposed to the normal renal function observed in the low-moderate risk group. However, further studies are needed to explore the role of bioactive sclerostin across different eGFR ranges in patients with type 2 diabetes.

Additionally, our analysis found significant differences in age, duration of diabetes, and systolic blood pressure between the high-very high cardiovascular risk group and the low-moderate risk cardiovascular risk group. Previous research suggests that age is a critical risk factor for cardiovascular disease, with its impact being influenced by various factors such as metabolic disturbances, diabetes, and traditional risk factors [30–32]. Our findings also revealed that age was independently associated with serum levels of bioactive sclerostin and periostin. This is in line with previous research indicating that serum bioactive sclerostin levels are correlated with age, potentially due to skeletal remodeling or imbalances in vascular remodeling that occur with aging [9]. Additionally, some studies have shown that higher plasma periostin levels are associated with poorer physical and cognitive capacities in older adults [33]. In contrast, other studies suggest that periostin expression declines with age [34]. Further research is therefore needed to clarify the relationship between periostin and aging. Regarding our findings on duration of diabetes, previous studies show that longer duration of diabetes is associated with a higher risk of cardiovascular disease and mortality [35, 36]. In addition, systolic blood pressure is known to be an important risk factor for cardiovascular disease [37], which is consistent with our results. We also found that serum levels of bioactive sclerostin and periostin were independently associated with systolic blood pressure. This may be explained by the association between higher blood pressure and cardiovascular disease CVD [38], as these proteins are also associated with increased cardiovascular risk.

On the other hand, our results demonstrated a positive correlation between serum levels of bioactive sclerostin and periostin with SCORE2-Diabetes algorithm. These findings are consistent with previous studies that have shown these bone proteins to be associated with cardiovascular events and mortality [9, 20, 39, 40]. Additionally,

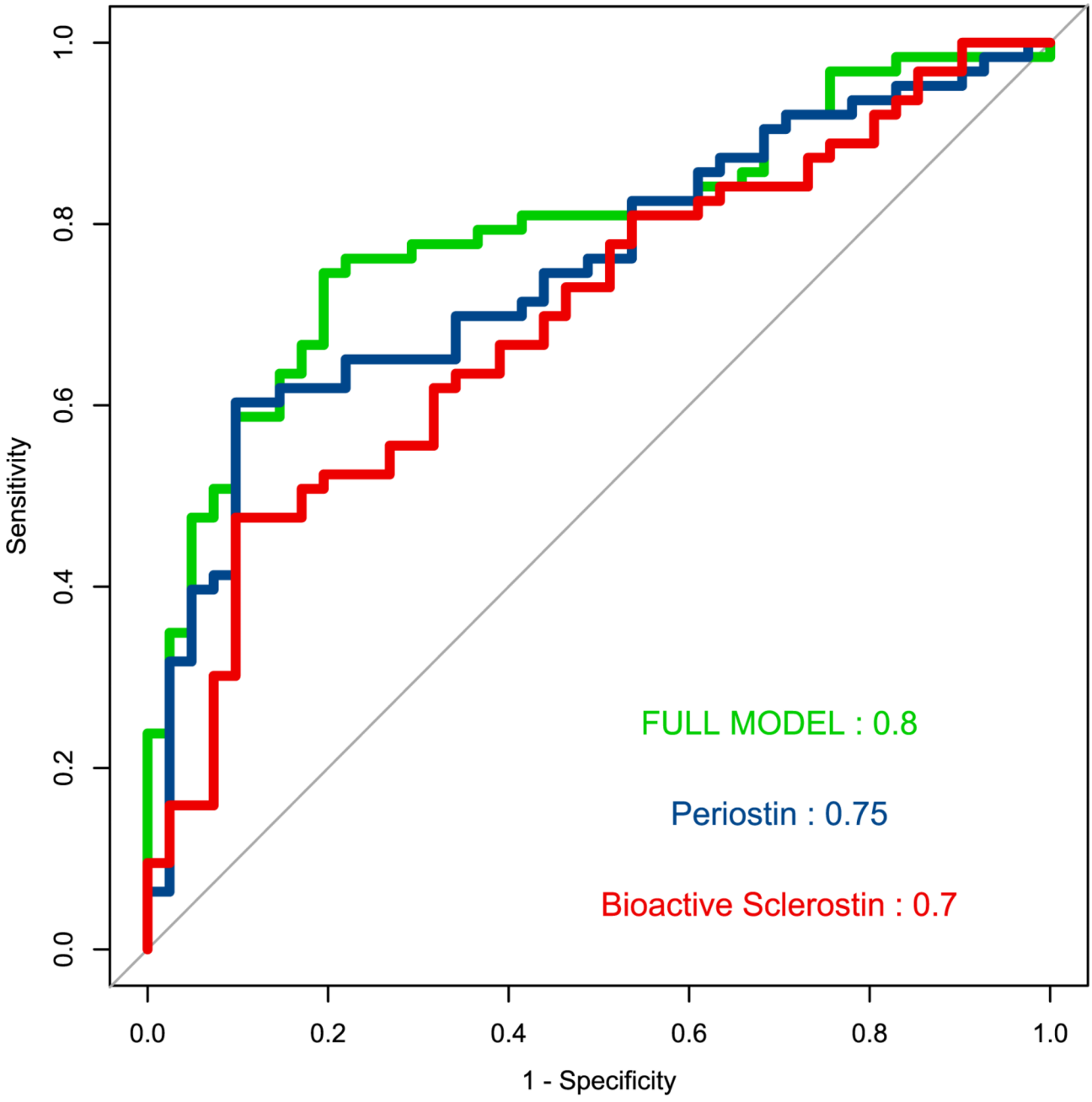


Fig. 3 ROC curve for the usefulness of serum levels of bioactive sclerostin and periostin in estimating cardiovascular risk based on SCORE2-Diabetes. In red, serum bioactive sclerostin levels; AUC = 0.6965 ($p = 0.001$). In blue, serum periostin levels; AUC = 0.7499 ($p < 0.001$). In green, full model (serum periostin and bioactive sclerostin levels); AUC = 0.7952 ($p < 0.001$). The AUC indicates the probability to predict an event and the values greater than 0.70 indicate a good predictive performance. CVD: cardiovascular disease; ROC: receiver operating characteristic; AUC: area under the curve

we observed increase in serum levels of bioactive sclerostin in patients with type 2 diabetes at high-very high risk of CVD according to SCORE2-Diabetes, mainly in males, aligns with prior research and suggests a potential involvement of sclerostin in vascular pathology. Previous studies have identified a positive association between serum sclerostin levels and subclinical atherosclerosis [41], atherosclerotic lesions [9, 39], and cardiovascular mortality [12] in the type 2 diabetes population, regardless of sex. Generally, circulating sclerostin levels are higher in men, a pattern observed in type 2 diabetes. These findings are consistent with numerous studies involving patients with type 2 diabetes [9, 10, 12] and healthy subjects [10, 42, 43]. Mödder et al. reported than the larger skeletal size in men may explain the gender differences in circulating sclerostin production and release

[42]. Regarding periostin, Luo Y. et al. found that patients with type 2 diabetes and obesity had significantly higher periostin levels [40]. Moreover, periostin has been shown to be associated in patients with myocardial fibrosis and heart failure [20]. Additionally, it has been suggested that periostin is important in adverse cardiac remodeling, especially in diabetic cardiomyopathy, leading to left ventricular remodeling and metabolic issues in diabetes. Elevated periostin expression has been observed in the hearts of diabetic rats, indicating its role in diabetic cardiomyopathy development [18], highlighting its connection to CVD. These studies are consistent with our results, which showed increased serum periostin levels in patients with type 2 diabetes at high-very high cardiovascular risk according to the SCORE2-Diabetes scale. However, until now, the relationship of these bone proteins with validated vascular risk algorithms had not been studied.

Interestingly, in our study, no association was found between circulating levels of ucOC and cardiovascular risk assessed by the SCORE2-Diabetes algorithm. This is in contrast to previous studies suggesting that ucOC is a marker of cardiovascular risk and type 2 diabetes [44–46]. The lack of association in our study may be attributed to differences in study design, population or the specific cardiovascular risk scale used.

Finally, we analyzed the diagnostic value of bioactive sclerostin and periostin for increased vascular risk using ROC curve analysis. The model combining both bone proteins demonstrated a substantial AUC of 0.774, indicating an enhanced predictive ability for vascular risk. Furthermore, specific threshold values for bioactive sclerostin and periostin (>131 pmol/L and >1144 pmol/L, respectively) were identified as indicative of high-very high risk (score≥10%) according to the SCORE2-Diabetes algorithm. Thus, bioactive sclerostin and periostin emerge as promising biomarkers for assessing cardiovascular risk in patients with type 2 diabetes. While these bone proteins show potential, their predictive value in clinical settings remains modest, highlighting the need for further research to validate their utility. In addition, it's important to consider that the use of anti-osteoporotic drugs, particularly romosozumab, may alter serum levels of these bone proteins [47, 48], potentially affecting their accuracy in predicting risk.

Our study presents some limitations. First, the cross-sectional design does not allow establishment of a cause-effect relationship. Moreover, our study population included only Spanish Caucasian individuals, from a specific area, and the use of common antihypertensive, antihyperlipidemic and antidiabetic drugs in patients may have influenced the results. In addition, the sample size is also a limitation that could reduce statistical power from the data analysis. Furthermore, this study did not consider subclinical atherosclerotic disease and heart failure with preserved ejection fraction (HF-pEF), which may have influenced the results. However, our work has several strengths. Our cross-sectional study presents an exhaustive evaluation of clinical, anthropometric, and biochemical parameters, integrating all variables that could influence cardiovascular risk. In addition, we performed rigorous statistical analyses, in order to obtain reliable results. Furthermore, for the first time, the relationship between bone proteins and vascular risk assessment algorithms is described and we postulate the diagnostic usefulness of sclerostin and periostin in the assessment of vascular risk in type 2 diabetes using the SCORE2- Diabetes. Finally, we measured bioactive sclerostin, with a specific antibody kit targeting the receptor binding region and rigorously validated for clinical samples according to guidelines: Food and Drug Administration (FDA)/ International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH)/ European Medicines Agency (EMEA).

Conclusion

The results of our study indicate an association between bioactive sclerostin and periostin with SCORE2-Diabetes algorithm, suggesting their potential usefulness as diagnostic biomarkers of vascular risk in patients with type 2 diabetes. Future prospective studies could validate the importance of these bone proteins in the assessment of vascular risk in the diabetic population.

Abbreviations

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BMI	Body mass index
CKD	Chronic kidney disease
CVD	Cardiovascular disease
ECM	Extracellular matrix
eGFR	Estimated glomerular filtration rate
ESC	European Society of Cardiology
FPG	Fasting plasma glucose
FRS	Framingham Risk Score
HbA1c	Glycated haemoglobin
HDL-c	High-density lipoprotein cholesterol
HF-pEF	Heart failure with preserved ejection fraction
LDL-c	Low-density lipoprotein cholesterol
TG	Triglycerides
ucOC	Undercarboxylated osteocalcin
ULN	Upper Limit of Normal

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Author contributions

M. M-T, A. G-M and S. G-S conceived and designed research; S. G-S, B. G-F and C. G-F performed experiments; M. M-T, A. G-M, L. M-H and S. G-S analyzed data; M. M-T, A. G-M interpreted results of experiments; S. G-S and L. M-H prepared figure; A. G-M, S. G-S, and M. M-T drafted manuscript; A. G-M, S. G-S and M. M-T edited and revised manuscript; M. M-T and C. G-F approved final version of manuscript. B.G-F, C. G-F and M. M-T funding acquisition. All listed authors commented on multiple versions of the manuscript and supported the

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Data availability
The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was conducted with the approval of the Ethics Committee of the University Hospital Clínico San Cecilio of Granada and conformed to the principles of the World Medical Association Declaration of Helsinki (Project ID:0858-N-17, Research Ethics Committee of Granada Center (CEI-Granada) on 26 April 2017). Informed consent was obtained from all subjects involved in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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