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RESEARCH ARTICLE

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Association of osteocalcin, osteoprotegerin, and osteopontin with cardiovascular disease and retinopathy in type 2 diabetes

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Abstract

Background: Novel biomarkers of vascular disease in diabetes could help identify new mechanistic pathways. Osteocalcin, osteoprotegerin, and osteopontin are key molecules involved in bone and vascular calcification processes, both of which are compromised in diabetes. We aimed to evaluate possible associations of osteocalcin, osteoprotegerin, and osteopontin with cardiovascular disease (CVD) and diabetic retinopathy (DR) among people with type 2 diabetes (T2D).

Materials and Methods: Osteocalcin, osteoprotegerin, and osteopontin concentrations were measured at enrolment in 848 participants with T2D from the Sapienza University Mortality and Morbidity Event Rate (SUMMER) Study (ClinicalTrials.gov: NCT02311244). Logistic regression models and propensity score matching were used to assess possible associations of osteocalcin, osteoprotegerin, and osteopontin with a history of CVD and with evidence of any grade of DR adjusting for confounders.

Results: Previous CVD was reported in 139 (16.4%) participants, while 144 (17.0%) had DR. After adjusting for possible confounders, osteocalcin but not osteoprotegerin or osteopontin concentrations were associated with a history of CVD (Odds Ratio [OR] and 95% CI for one standard deviation (SD) increase in osteocalcin concentrations (natural log): 1.35 (1.06–1.72), $p = 0.014$). Associations with prevalent DR were seen for osteoprotegerin (OR for one SD increase in osteoprotegerin concentrations (natural log): 1.25 (1.01–1.55), $p = 0.047$) and osteopontin (OR for one SD increase in osteopontin concentrations (natural log): 1.25 (1.02–1.53), $p = 0.022$), but not osteocalcin.

Conclusions: In T2D, higher serum osteocalcin concentrations are associated with macrovascular complications and higher osteoprotegerin and osteopontin concentrations with microvascular complications, suggesting that these osteokines might be involved in pathways directly related to vascular disease.

KEYWORDS
biomarkers, bone-vascular axis, cardiovascular disease, diabetic retinopathy, osteokines, type 2 diabetes

1 | INTRODUCTION

Complications of diabetes mellitus, both cardiovascular disease (CVD) and microvascular outcomes, such as end-stage renal disease and diabetic retinopathy (DR), are major causes of morbidity and mortality worldwide.[1,2] The identification of new potential pathways for diabetic complications may help find new targets for therapies, whilst new biomarkers could improve risk stratification. In this regard, there is increasing evidence supporting the existence of a bone-vascular axis,[3] and identifying bone metabolism markers related to vascular disease is currently an open field of research. Among such biomarkers, osteocalcin, osteoprotegerin, and osteopontin have shown actions strictly related to cardiometabolic pathways. Osteocalcin is involved in the regulation of beta-cell proliferation, insulin secretion, and sensitivity.[4] Moreover, osteocalcin has been shown to be expressed in the calcific atherosclerotic lesions and the vascular smooth muscle cells (SMC) of the vessel wall, suggesting a potential role in the differentiation of vascular SMC into osteogenic cells.[5]

We have recently shown that osteoprotegerin, a master regulator of osteoclast differentiation and function, is independently associated with advanced atherosclerosis in people without diabetes[6] and with worse metabolic profile and presence of carotid atherosclerosis in a small group of people with type 2 diabetes.[7] Osteoprotegerin is a member of the tumour necrosis factor (TNF) superfamily that inhibits osteoclastic bone resorption through its presentation as a 'decoy receptor' for receptor activator of nuclear factor κB ligand (RANKL)[8] inducing higher bone mineral density. The role of osteoprotegerin in vascular health remains controversial, but high serum osteoprotegerin concentrations have been associated with the presence of vascular calcifications,[9] atherosclerosis,[10] and cardiovascular mortality.[11]

Some studies also suggest that osteopontin, a structural glycoprotein of the bone matrix, may play a role in regulating vascular calcification, even though evidence in this regard is contrasting.[7,12]

As the role of osteokines as markers of CVD in T2D remains unclear and little is known about their possible association with microvascular complications, we hypothesised that osteocalcin, osteoprotegerin, and osteopontin may be independent markers of diabetic complications. Since the pathogenesis of CVD and DR is sustained by different molecular and cellular mechanisms,[13] we also hypothesised that different osteokines, based on their different biology, may be differentially associated with these two complications of diabetes. Accordingly, we analysed data from the 'Sapienza University Mortality and Morbidity Event Rate (SUMMER) study in diabetes' to evaluate possible associations between circulating bone metabolism markers and macro- and micro-vascular diabetic complications.

2 | MATERIALS AND METHODS

2.1 | Study design and population

This is a cross-sectional analysis performed on the baseline data and serum samples collected within the SUMMER study (registered at ClinicalTrials.gov, NCT02311244). This was an observational, prospective, collaborative study aimed at identifying new predictors of all-cause and cardiovascular mortality and morbidity in patients with adult-onset diabetes.[14] SUMMER enroled consecutive individuals, regardless of being newly or previously diagnosed with T2D, from July 2014 to December 2018 in 10 Italian diabetes clinics. Exclusion criteria included severe psychiatric illnesses, end-stage renal disease, renal dialysis, hepatic cirrhosis, active cancer of any type, and chronic treatment with corticosteroids.

At the time of enrolment, demographic data, medical history, and biochemical information as well as blood samples were collected from each participant. Serum samples were subsequently aliquoted and stored at −80°C prior to assay.

At the time the population for this study was selected (March 2019), 2486 SUMMER participants had been screened for the presence of glutamic acid decarboxylase antibodies (GADA). We aimed to analyse a subgroup comprising the first 850 GADA-negative SUMMER participants. However, two participants were excluded because technical issues meant their samples were not available for the measurement of osteocalcin, osteoprotegerin and osteopontin, resulting in a final study cohort of 848 individuals.

2.2 | Laboratory assays and data extraction

Commercial BioVendor ELISA kits were used to assess serum concentrations of osteocalcin (Brno, Czech Republic; Catalogue # RIS002R), osteoprotegerin (Brno, Czech Republic; Catalogue #

RD194003200), and osteopontin (Brno, Czech Republic; Catalogue # RD191446200R). The osteocalcin inter-assay coefficient of variation is 4.6% with an intra-assay coefficient of variation being 3.9%, the osteoprotegerin inter-assay coefficient of variation is 5.8% with an intra-assay coefficient of variation being 3.5%, and the osteopontin inter-assay coefficient of variation is 3.9% with an intra-assay coefficient of variation being 5.7%.

The following data were retrieved from the SUMMER study database for the 848 selected participants:

- Anthropometric parameters: age, sex, body mass index (BMI), and systolic and diastolic blood pressure.
- Medical history: age at diabetes diagnosis, duration of illness, smoking habit, therapy for diabetes, therapy for dyslipidemia, therapy for hypertension, and history of myocardial infarction or stroke.
- Biochemical parameters: glycated haemoglobin (HbA_{1c}), total cholesterol, calculated LDL-cholesterol, HDL-cholesterol, triglycerides, uric acid, and serum creatinine.

The estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (EPI-CKD) equation.[15] The presence of hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or if the participant was taking any antihypertensive treatment (angiotensin converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, beta-blockers, or alpha blockers), at the time of enrolment. Presence of dyslipidemia was defined as LDL-cholesterol >100 mg/dl, HDL-cholesterol <40 mg/dl, triglycerides >200 mg/dl, or if the participant was taking statins or fibrates at the time of enrolment.

2.3 | Outcomes

The two outcomes we analysed were history of CVD (defined as history of acute myocardial infarction, coronary artery revascularisation, or stroke) and prevalent DR (defined as a fundus oculi examination performed by an expert ophthalmologist documenting any grade of retinopathy or maculopathy in either eye). Since osteoprotegerin and osteocalcin are excreted in urine, diabetic nephropathy was not investigated as a complication, but eGFR was included as a possible confounder.

2.4 | Statistical analysis

Descriptive statistics are presented as frequencies and proportions for categorical variables, and as mean and standard deviation or median and 25th-75th percentiles, as appropriate, for continuous variables. The normal distribution assumption for continuous variables was tested with the Shapiro-Wilk test. Continuous variables with a parametric distribution were compared between groups using Student's *t* test, and the Kruskal–Wallis test was used for non-parametric variables. Spearman rank test was used to test relationships between continuous variables. Categorical variables were compared with a χ^2 or Fisher's exact test as appropriate. Standardized mean difference (SMD) between groups was also calculated. Logistic regression models with the study outcomes as dichotomous dependent variables and osteocalcin, osteoprotegerin or osteopontin as main exposures were used to estimate the odds ratios (OR) with 95% confidence intervals (CI) associated with a one standard deviation increase in the serum concentrations (natural log) of the main exposures after adjusting for potential confounders. Since the pathophysiology and the mechanisms of injury of CVD and DR differ,[13] we analysed CVD and DR as separate outcomes. Five separate models were evaluated: (1) adjusted for general confounders (sex, age, smoking habits and BMI); (2) adjusting as for model 1 plus diabetes-related variables (HbA_{1c}, age at diagnosis); (3) adjusting as for model 2 plus CV comorbidities (uric acid, dyslipidemia, hypertension); (4) adjusting as for model 3 plus eGFR; and (5) adjusted as for model 4 plus glucose-lowering medications (categorised as: 'diet alone', 'non-insulin therapy' or 'insulin therapy with or without other anti-diabetes drugs'). Osteocalcin, osteoprotegerin, and osteopontin were jointly tested in the model if associated with the outcome at a conservative *p*-value <0.1. CVD was not included as a possible confounder in the model having DR as an outcome, and vice versa, because the two complications were not overlapping in our population (i.e. were not associated at a conservative *p*-value <0.1) The following non-parametric variables were logarithmically transformed (natural log) before entering the model: osteocalcin, osteoprotegerin, osteopontin, age, BMI, age at diagnosis, HbA_{1c}, uric acid, and eGFR.

Propensity score matching with Kernel matching was also used to test differences in osteokines levels between people with or without CVD or DR matched for unbalanced clinical features (see Supplementary Appendix for details).[16]

Associations of each bone biomarker with the two study outcomes were tested at a two-sided alpha-level <0.05 after Bonferroni correction (i.e. after multiplying *p*-values for the number of tests). Assuming a distribution of bone biomarkers in the population similar to our previous observations,[6,17] the study was >80% powered to detect a 15% difference in bone biomarkers between groups, at an alpha-level of 0.05. Stata/IC 12.1 (StataCorp) and Prism 9.0 (GraphPad Software) were used to perform the statistical analyses and produce graphical representations.

2.5 | Ethics

The study was performed in accord with the Declaration of Helsinki. The study protocol was approved by the coordinating centre's Ethic Committee (Comitato Etico 'Sapienza', from the Umberto I 'Sapienza' University Hospital, in Rome, Prot. *n.* 782/2014) and thereafter by the Ethics Committee of each centre outside the Umberto I 'Sapienza'

University Hospital. Participants signed a written informed consent to participate in the study.[14]

3 | RESULTS

3.1 | Population features

The 848 participants had a median [25th, 75th percentiles] age of 71 [64, 78] years, diabetes duration of 10 [5, 16] years and 348 (41.0%) were female. At the time of enrolment, median HbA_{1c} was 6.8 [6.1, 7.8] %, eGFR 81.1 (64.5, 93.1) ml/min/1.73 m,[2] and 146 (17.2%) were smokers. A history of CVD was reported by 139 (16.4%) participants, while 144 (17.0%) had prevalent DR. Overall, median osteocalcin concentration was 3.9 [1.6, 8.5] ng/ml, osteopontin 2.9 [2.1, 3.9] ng/ml and osteoprotegerin 10.3 [7.1, 17.2] pmol/L. The three osteokines were not significantly related to each other (osteocalcin vs. osteopontin: $r = -0.0037$, $p = 0.91$; osteocalcin vs. osteoprotegerin: $r = 0.058$, $p = 0.090$; osteoprotegerin vs. osteopontin: $r = 0.06$, $p = 0.080$). Osteoprotegerin levels were higher among females than males ($p < 0.001$), while no differences in osteocalcin and osteopontin levels by sex were found (Supplementary Table S1). Osteocalcin and osteoprotegerin levels tend to increase with age, age at diagnosis, disease duration, and lower eGFR (Supplementary Table S1). HbA_{1c} was directly related to osteoprotegerin concentrations, while inverse associations between BMI and osteocalcin and between triglycerides and osteocalcin were found (Supplementary Table S1).

3.2 | Cardiovascular disease

People with a history of CVD, compared with those without, were more frequently male, older at diabetes onset, with a longer diabetes duration at enrolment, and less frequently never smokers (Table 1).

TABLE 1 Population features by positive history or not of cardiovascular disease.

	Cardiovascular disease			
	No *n* = 709	Yes *n* = 139	SMD	*p* value
Females, *n* (%)	309 (43.6)	39 (28.1)	0.328	0.001
Age, years	70 [63–77]	76 [69–81]	−0.517	<0.001
Age at diagnosis, year	55 [47–62]	59 [49–66]	−0.211	0.014
Diabetes duration, years	10 [5–16]	11 [6–18]	−0.230	0.015
BMI, kg/m^2	29.1 [26.0–32.8]	29.4 [26.0–32.2]	0.045	0.55
Smokers, *n* (%)			0.419	<0.001
- Never	313 (44.1)	40 (28.8)		
- Ex	268 (37.8)	81 (58.3)		
- Current	128 (18.1)	18 (13.0)		
eGFR, ml/min/1.73 m^2	82.8 [66.8–94.0]	70.1 [54.1–84.4]	0.489	<0.001
HbA_{1c}, %	6.8 [6.1–7.8]	6.9 [6.3–8]	−0.106	0.075
Total cholesterol, mg/dL	169 [147–192]	150 [136–176]	0.343	<0.001
LDL-cholesterol, mg/dL	92.6 [73.8–113.8]	80.4 [64.4–101.4]	0.267	<0.001
HDL-cholesterol, mg/dL	47 [39–56]	43 [38–51]	0.239	0.022
Triglycerides, mg/dL	122 [91–170]	126 [95–168]	0.068	0.72
Vitamin D, ng/mL	24.1 [17.9–32.5]	23.75 [17.9–29.7]	0.132	0.32
Uric acid, mg/dL	5.4 [4.5–6.4]	5.6 [5–6.7]	−0.221	0.018
History of hypertension, *n* (%)	603 (85.1)	134 (96.4)	0.399	<0.001
History of dyslipidaemia, *n* (%)	638 (89.9)	135 (97.1)	0.294	0.007
Glucose-lowering treatment strategy, *n* (%)			0.221	0.056
Only diet	55 (7.8)	14 (10.1)		
Non-insulin therapy	454 (64.0)	74 (53.2)		
Insulin therapy	200 (28.2)	51 (36.7)		

Note: SMD, standardized mean difference. Values are median [25–75th percentile] for continuous variables and number (percentages) for categorical variables.

Their eGFR was lower, their HbA_{1c} and uric acid concentrations higher, and they more frequently had hypertension and dyslipidemia (Table 1). Insulin therapy was slightly more frequently used among people with a history of CVD. There was no difference in the prevalence of DR between people with and without CVD (18.7% vs. 16.6%, $p = 0.55$).

Osteocalcin and osteoprotegerin concentrations were higher in people with than in those without CVD (osteocalcin: 5.5 [2.6–10.0] vs. 3.7 [1.4–8.2] ng/ml, Bonferroni-adjusted *p*-value: 0.0012; osteoprotegerin: 11.5 [8.2–20.1] vs. 10.0 [7.0–16.4] pmol/L, Bonferroni-adjusted *p*-value: 0.021), while no differences in osteopontin concentrations were observed (2.9 [2.1–4.0] vs. 2.8 [2.1–3.9], Bonferroni-adjusted *p*-value: 0.99). After adjustment for all confounders, osteocalcin, but not osteoprotegerin, concentrations remained associated with CVD (Figure 1).

Levels of osteocalcin remained associated with CVD also when groups of people with and without CVD were matched for cardiovascular risk factors with propensity score matching (Supplementary Appendix).

3.3 | Diabetic retinopathy

People with DR, compared with those without DR, were older with longer diabetes duration and younger age at diagnosis (Table 2). Their median HbA_{1c} was higher, while median eGFR was lower. No differences in the prevalence of dyslipidemia and hypertension were found. People with DR were more frequently treated with insulin. There was no difference in the prevalence of CVD between people with and without DR (18.1% vs. 16.1%, $p = 0.55$).

People with DR had higher osteoprotegerin values (13.2 [8.8–22.3] vs. 9.8 [6.8–15.9] pmol/L, Bonferroni-adjusted *p*-value: <0.001) and osteopontin values (3.1 [2.3–4.6] vs. 2.8 [2.1–3.8] ng/ml, Bonferroni-adjusted *p*-value: 0.039) compared to people without DR. Adjusting for confounders did not alter these associations (Figure 2). Conversely, no significant difference in osteocalcin values was found between those with or without DR (4.6 [1.8–8.8] vs. 3.8 [1.6–8.5], Bonferroni-adjusted *p*-value: 0.32).

Levels of both osteoprotegerin and osteopontin remained associated with DR also when groups of people with and without DR were

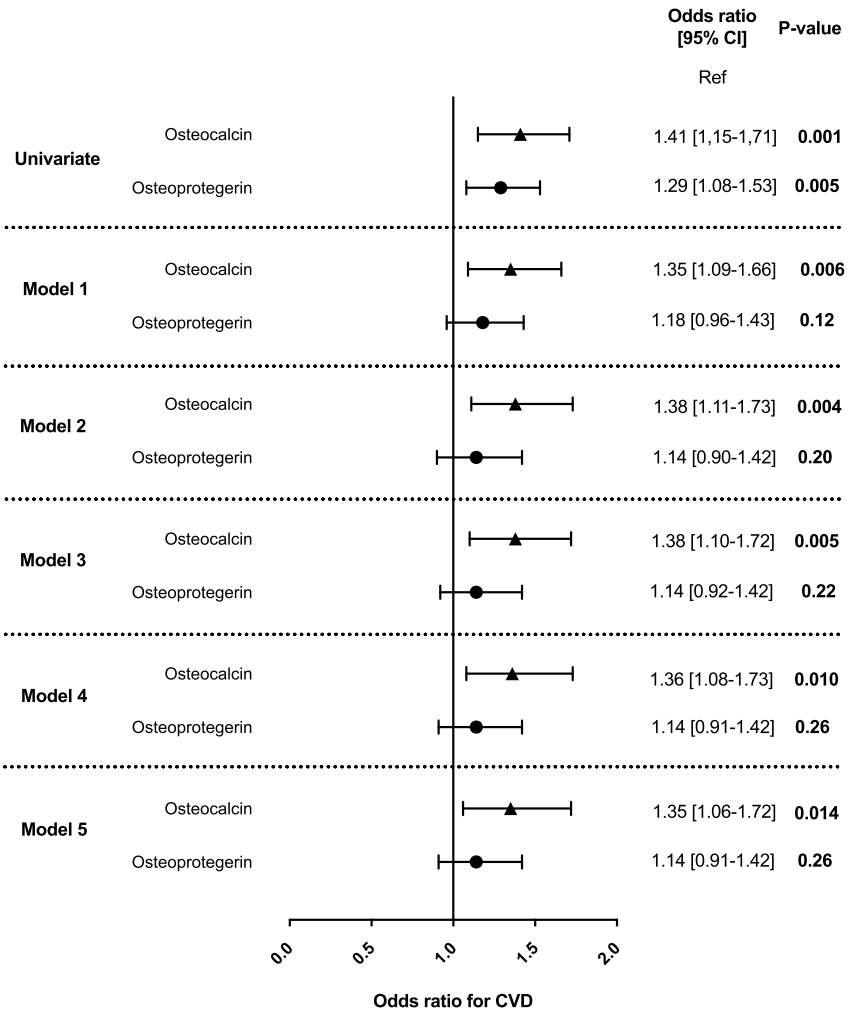

FIGURE 1 Forest plot of regression models testing the association of osteocalcin and osteoprotegerin with a history of CVD. Odds ratio (OR) and 95% confidence intervals (CI) are for one standard deviation increase in osteocalcin or osteoprotegerin concentrations (natural log). Osteopontin was not included in the models because it was not associated with CVD in the pairwise comparison (see text). Model 1: adjusted for sex, age, smoking habits, and BMI. Model 2: adjusted as in model 1 plus HbA_{1c} and age at diabetes diagnosis. Model 3: adjusted ad in model 2 plus uric acid, dyslipidemia, and hypertension. Model 4: adjusted as in model 3 plus eGFR. Model 5: adjusted as in model 4 plus glucose-lowering treatment strategy.

TABLE 2 Population features by presence or not of diabetic retinopathy.

	Diabetic retinopathy			
	No $n = 704$	Yes $n = 144$	SMD	p value
Females, *n* (%)	287 (40.8)	61 (42.4)	0.032	0.72
Age, years	71 [64–77]	72.5 [66–79]	−0.243	0.023
Age at diagnosis, year	57 [49–63]	51 [44–58.5]	0.426	<0.001
Diabetes duration, years	9 [4–15]	15 [10–24]	−0.736	<0.001
BMI, kg/m^2	29.1 [25.9–32.5]	30.3 [26.2–33.5]	−0.107	0.17
Smokers, *n* (%)			0.134	0.37
- Never	290 (41.2)	63 (43.8)		
- Ex	287 (40.8)	62 (43.0)		
- Current	127 (18.0)	19 (13.2)		
eGFR, ml/min/1.73 m^2	82.3 [65.8–93.9]	74.6 [59.5–88.4]	0.309	0.001
HbA_{1c}, %	6.7 [6.1–7.6]	7.5 [6.6–8.5]	−0.492	<0.001
Total cholesterol, mg/dL	166 [145–191]	166 [142.5–192]	0.033	0.72
LDL-cholesterol, mg/dL	89.8 [73.2–111.2]	91.6 [69.7–114.3]	−0.064	0.77
HDL-cholesterol, mg/dL	46 [39–55]	44.5 [38–54.5]	0.117	0.25
Triglycerides, mg/dL	122 [91–171]	124 [96.5–162.5]	0.159	0.82
Vitamin D, ng/mL	24.2 [18.3–32.5]	23.3 [15.5–29]	0.289	0.007
Uric acid, mg/dL	5.5 [4.6–6.5]	5.5 [4.5–6.4]	0.041	0.66
History of hypertension, *n* (%)	608 (86.4)	129 (89.6)	0.099	0.30
History of dyslipidaemia, *n* (%)	642 (91.2)	131 (90.9)	0.008	0.93
Glucose-lowering treatment strategy, *n* (%)			0.720	<0.001
Only diet	64 (9.1)	6 (3.5)		
Non-insulin therapy	471 (66.9)	57 (39.6)		
Insulin therapy	169 (24.0)	82 (56.9)		

Note: SMD, standardized mean difference. Values are median [25–75th percentile] for continuous variables and number (percentages) for categorical variables.

matched with propensity score matching for clinical features significantly differing between these two groups in the whole population (Supplementary Appendix).

4 | DISCUSSION

This cross-sectional analysis performed on baseline SUMMER study data shows that, among people with T2D, serum osteocalcin concentrations are positively associated with CVD and that osteoprotegerin and osteopontin concentrations are positively associated with DR. More specifically, for each standard deviation increase in serum osteocalcin concentrations (natural log), we found 32% higher probability of having CVD. Additionally, each standard deviation increase in osteoprotegerin and osteopontin concentrations (natural log) was associated with 25% higher probability of having DR.

These results suggest that osteokines may be involved in pathways directly related to vascular disease, expanding their biological relevance.

Several previous studies have investigated the association of osteocalcin with CVD.[18] However, these studies invariably included a heterogeneous population with low proportion of people with T2D. Since a complex relation between osteocalcin and diabetes has been described previously,[19,20] our study aimed at validating the association between osteocalcin and CVD in a large contemporary cohort comprising only people with non-autoimmune diabetes ($n = 848$). In this cohort, higher osteocalcin concentrations were associated with the presence of CVD after multivariable adjustments. Similar analyses in a general population reported conflicting results with osteocalcin being either directly or inversely associated with existing CVD.[21] A partial explanation for such inconsistencies in the literature may be that serum osteocalcin concentrations are influenced by several factors such as ethnicity, sex, menopausal status, and

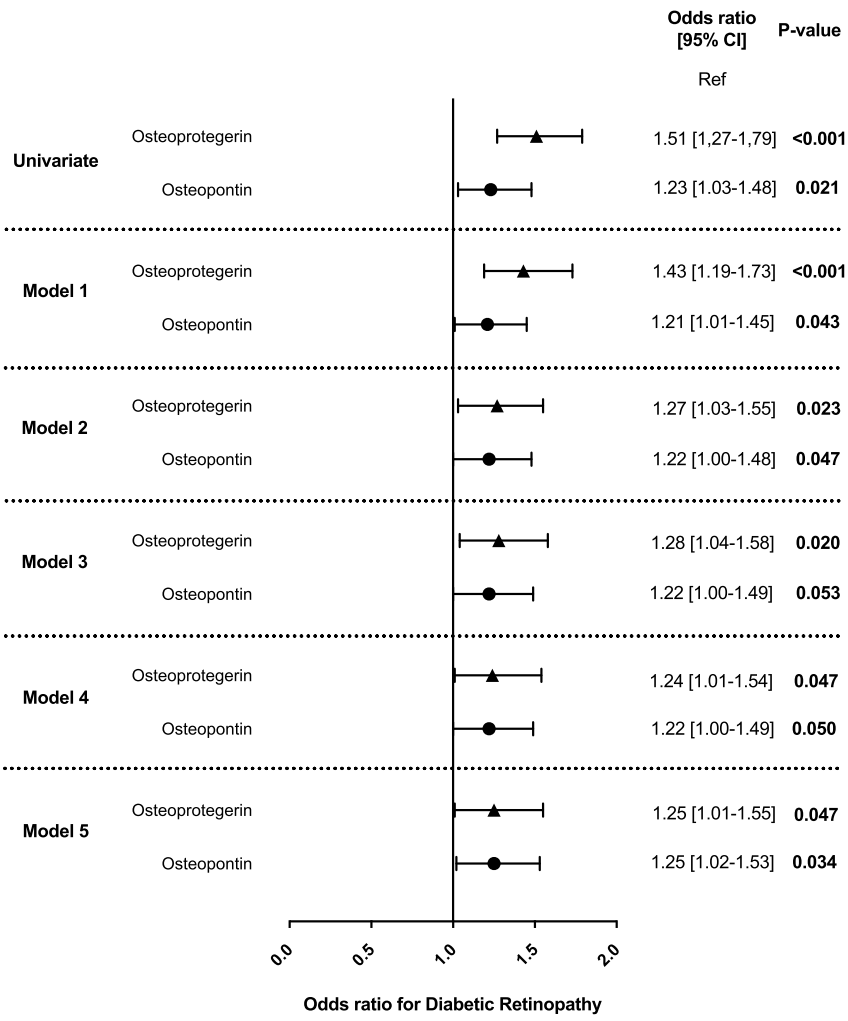

FIGURE 2 Forest plot of the regression models testing the associations of osteoprotegerin and osteopontin with DR. Odds ratio (OR) and 95% confidence intervals (CI) are given for one standard deviation increase in osteoprotegerin and osteopontin concentrations (natural log). Osteocalcin was not included in the models because it was not associated with DR in the pairwise comparison (see text). Model 1: adjusted for sex, age, smoking habits, and BMI. Model 2: adjusted as in model 1 plus HbA_{1c} and age at diagnosis. Model 3: adjusted ad in model 2 plus uric acid, dyslipidemia, and hypertension. Model 4: adjusted as in model 3 plus eGFR. Model 5: adjusted as in model 4 plus glucose-lowering treatment strategy.

diabetes.[22] Accordingly, our study attempted to minimise the effect of confounders on the relationship between osteocalcin and CVD by focussing exclusively on a well-characterised cohort of people with T2D.

As with previous observations in people without diabetes[6] and those with type 1 and type 2 diabetes,[7,23] we report higher osteoprotegerin concentrations among SUMMER participants with CVD than their non-CVD counterparts. However, this association disappeared after adjusting for general confounders, suggesting that the relationship between osteoprotegerin and CVD in T2D may be mediated by common cardiovascular risk factors or therapies. On the contrary, we found an independent association between osteoprotegerin and DR, which is in line with previous smaller studies suggesting higher osteoprotegerin concentrations in people with diabetes with proliferative retinopathy than in those with non-proliferative or no retinopathy.[24] A proposed mechanism involves the dysregulation of the osteoprotegerin/RANKL/RANK pathway that leads to inflammation and angiogenesis in proliferative DR.[25] We also observed that a one standard deviation increase in osteopontin concentrations (natural log) increases by 25% the risk of retinopathy. These results are supported by previous studies investigating molecular mechanisms underlying DR. Specifically, Zhang et al. reported that osteopontin induces angiogenesis in patients with diabetes with proliferative retinopathy.[26] Our study corroborates these observations on a large sample of 848 people with T2D and provides a more robust and clinically relevant evidence of the associations of osteoprotegerin and osteopontin with DR.

Taken together, the implications of our findings are two-fold. On the one hand, osteocalcin, osteoprotegerin and osteopontin levels appear as additional independent risk factors for vascular complications of T2D. On the other hand, the differential association, especially of osteocalcin and osteopontin, with macrovascular and microvascular complications may suggest different mechanisms of action, which should be addressed in future studies.

Strengths of this study include the multi-institutional contemporary cohort, the large sample size, the centralised serum analysis, and the exclusion of patients testing positive for GADA.

A limitation of our study is that, as with many previous studies investigating the effect of bone biomarkers, our analysis relied on a cross-sectional design that cannot establish a cause-and-effect relationship between bone biomarkers and clinical outcomes. A prospective evaluation of the longitudinal data from the SUMMER study

in diabetes is planned and will be performed once the follow-up of all participants is terminated. Furthermore, we acknowledge that we observed CVD and DR in a relatively small proportion of the population, which might have had an effect on the width of the confidence intervals of the described associations.

In conclusion, our study highlights the relationships between bone biomarkers and vascular complications in individuals with diabetes. Specifically, higher osteocalcin concentrations were associated with a greater likelihood of a history of CVD, and higher osteoprotegerin and osteopontin concentrations were associated with a higher likelihood of prevalent DR.

AUTHOR CONTRIBUTIONS

Ernesto Maddaloni designed the study, analysed and interpreted data and wrote the first draft of the manuscript; LC contributed to the design of the study, data acquisition, interpretation and manuscript writing; RA performed laboratory measurements and contributed to data interpretation; Marco G. Baroni, Maria G. Cavallo, Efisio Cossu, Paola D'Angelo, Luca D'Onofrio, Salvatore De Cosmo, Frida Leonetti, Susanna Morano, Lelio Morviducci, Nicola Napoli, Sabrina Prudente and Giuseppe Pugliese acquired data. Massimiliano Copetti, helped and supervised statistical analysis; Kyoungmin Park helped in data interpretation; Rury R. Holman contributed to data interpretation and revised the manuscript for important intellectual content; Vincenzo Trischitta contributed to data analysis and interpretation and revised manuscript for important intellectual content; Raffaella Buzzetti was responsible for the conception of the study, contributed to study design and data interpretation and revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

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CONFLICTS OF INTEREST STATEMENT

The authors declare no conflicts of interest related to this manuscript.

ETHICS STATEMENT

The study was performed in accord with the Declaration of Helsinki. The study protocol was approved by the coordinating centre's Ethic Committee (Comitato Etico 'Sapienza', from the Umberto I 'Sapienza' University Hospital, in Rome, Prot. *n.* 782/2014) and thereafter by the Ethics Committee of each centre outside the Umberto I 'Sapienza' University Hospital. Participants signed a written informed consent to participate in the study.[14]

DATA AVAILABILITY STATEMENT

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the SUMMER study group on reasonable request.

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PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/dmrr.3632.

REFERENCES

1. Harding JL, Pavkov ME, Magliano DJ, Shaw JE, Gregg EW. Global trends in diabetes complications: a review of current evidence. *Diabetologia*. 2019;62(1):3-16. https://doi.org/10.1007/s00125-018-4711-2
2. Dal Canto E, Ceriello A, Rydén L, et al. Diabetes as a cardiovascular risk factor: an overview of global trends of macro and micro vascular complications. *Eur J Prev Cardiol*. 2019;26(2_Suppl l):25-32. https://doi.org/10.1177/2047487319878371
3. Lampropoulos CE, Papaioannou I, D'Cruz DP. Osteoporosis--a risk factor for cardiovascular disease? *Nat Rev Rheumatol*. 2012;8(10):587-598. https://doi.org/10.1038/nrrheum.2012.120
4. D'Onofrio L, Maddaloni E, Buzzetti R. Osteocalcin and sclerostin: background characters or main actors in cardiovascular disease? *Diabetes Metab Res Rev*. 2020;36(1). https://doi.org/10.1002/DMRR.3217
5. Rashdan NA, Sim AM, Cui L, et al. Osteocalcin regulates arterial calcification via altered wnt signaling and glucose metabolism. *J Bone Min Res*. 2020;35(2):357-367. https://doi.org/10.1002/JBMR.3888
6. Del Toro R, Cavallari I, Tramontana F, et al. Association of bone biomarkers with advanced atherosclerotic disease in people with overweight/obesity. *Endocrine*. 2021;73(2):339-346. https://doi.org/10.1007/s12020-021-02736-8
7. Barchetta I, Ceccarelli V, Cimini FA, et al. Impaired bone matrix glycoprotein pattern is associated with increased cardio-metabolic risk profile in patients with type 2 diabetes mellitus. *J Endocrinol*

Invest. 2019;42(5):513-520. https://doi.org/10.1007/s40618-018-0941-x
8. Rochette L, Meloux A, Rigal E, Zeller M, Cottin Y, Vergely C. The role of osteoprotegerin and its ligands in vascular function. *Int J Mol Sci*. 2019;20(3):705. https://doi.org/10.3390/IJMS20030705
9. Fadini GP, Albiero M, Menegazzo L, et al. Procalcific phenotypic drift of circulating progenitor cells in type 2 diabetes with coronary artery disease. *Exp Diabetes Res*. 2012;2012:921685-921687. https://doi.org/10.1155/2012/921685
10. Kiechl S, Schett G, Wenning G, et al. Osteoprotegerin is a risk factor for progressive atherosclerosis and cardiovascular disease. *Circulation*. 2004;109(18):2175-2180. https://doi.org/10.1161/01.CIR.0000127957.43874.BB
11. Browner WS, Lui L.-Y, Cummings SR. Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. *J Clin Endocrinol Metab*. 2001;86(2):631-637. https://doi.org/10.1210/JCEM.86.2.7192
12. Steitz SA, Speer MY, McKee MD, et al. Osteopontin inhibits mineral deposition and promotes regression of ectopic calcification. *Am J Pathol*. 2002;161(6):2035-2046. https://doi.org/10.1016/S0002-9440(10)64482-3
13. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. *Cell Metab*. 2013;17(1):20-33. https://doi.org/10.1016/j.cmet.2012.11.012
14. Barchetta I, Capoccia D, Baroni MG, et al. The "Sapienza university mortality and morbidity event rate (SUMMER) study in diabetes": study protocol. *Nutr Metab Cardiovasc Dis*. 2016;26(2):103-108. https://doi.org/10.1016/J.NUMECD.2015.09.009
15. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*. 1999;130(6):461-470. https://doi.org/10.7326/0003-4819-130-6-199903160-00002
16. Jann B. Stata Module for Multivariate-Distance and Propensity-Score Matching, Including Entropy Balancing, Inverse Probability Weighting, (Coarsened) Exact Matching, and Regression Adjustment; 2017. Accessed 18 November 2022. https://ideas.repec.org/c/boc/bocode/s458346.html
17. Maddaloni E, D'Onofrio L, Lauria A, et al. Osteocalcin levels are inversely associated with Hba1c and BMI in adult subjects with long-standing type 1 diabetes. *J Endocrinol Invest*. 2014;37(7):661-666. https://doi.org/10.1007/s40618-014-0092-7
18. Seidu S, Kunutsor SK, Khunti K. Association of circulating osteocalcin with cardiovascular disease and intermediate cardiovascular phenotypes: systematic review and meta-analysis. *Scand Cardiovasc J*, 2019;53(6):286-295, https://doi.org/10.1080/14017431.2019.1655166
19. Darwish L, Nguyen MM, Saleem M, et al. Lower serum osteocalcin concentrations in patients with type 2 diabetes and relationships with vascular risk factors among patients with coronary artery disease. *J Diabetes Complicat*. 2019;33(5):390-397. https://doi.org/10.1016/J.JDIACOMP.2019.01.003
20. Sanchez-Enriquez S, Ballesteros-Gonzalez IT, Villafán-Bernal JR, et al. Serum levels of undercarboxylated osteocalcin are related to cardiovascular risk factors in patients with type 2 diabetes mellitus and healthy subjects. *World J Diabetes*. 2017;8(1):11. https://doi.org/10.4239/WJD.V8.I1.11
21. Pieralice S, Vigevano F, Del Toro R, Napoli N, Maddaloni E. Lifestyle management of diabetes: implications for the bone-vascular Axis. *Curr Diab Rep*. 2018;18(10):84. https://doi.org/10.1007/s11892-018-1060-y
22. Ma H, Lin H, Hu Y, et al. Serum levels of osteocalcin in relation to glucose metabolism and carotid atherosclerosis in Chinese middle-aged and elderly male adults: the Shanghai Changfeng Study. *Eur J Intern Med*. 2014;25(3):259-264. https://doi.org/10.1016/J.EJIM.2014.01.017
23. Gordin D, Soro-Paavonen A, Thomas MC, et al. Osteoprotegerin is an independent predictor of vascular events in Finnish adults with type 1 diabetes. *Diabetes Care*. 2013;36(7):1827-1833. https://doi.org/10.2337/dc12-2170
24. Yu G, Ji X, Jin J, Bu S. Association of serum and vitreous concentrations of osteoprotegerin with diabetic retinopathy. *Ann Clin Biochem*. 2015;52(2):232-236. https://doi.org/10.1177/0004563214533669
25. Abu El-Asrar AM, Ahmad A, Alam K, et al. Unbalanced vitreous levels of osteoprotegerin, RANKL, RANK, and TRAIL in proliferative diabetic retinopathy. *Ocular Immunol Inflamm*. 2017;26(8):1248-1260. https://doi.org/10.1080/09273948.2017.1343855
26. Zhang X, Chee WK, Liu S, et al. Association of plasma osteopontin with diabetic retinopathy in Asians with type 2 diabetes. *Mol Vis*. 2018;24:165.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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