

The Relationship Between Plasma Osteoprotegerin Levels and Coronary Artery Calcification in Uncomplicated Type 2 Diabetic Subjects

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- OBJECTIVES** This study sought to prospectively evaluate the relationship between plasma osteoprotegerin (OPG), inflammatory biomarkers (high-sensitivity C-reactive protein [hs-CRP], interleukin-6 [IL-6]), coronary artery calcification (CAC), and cardiovascular events in patients with type 2 diabetes.
- BACKGROUND** Arterial calcification is a prominent feature of atherosclerosis and is associated with an increased risk of cardiovascular events. Osteoprotegerin is a cytokine that has recently been implicated in the regulation of vascular calcification.
- METHODS** A total of 510 type 2 diabetic patients (53 ± 8 years; 61% male) free of symptoms of cardiovascular disease were evaluated by CAC imaging. Risk factors, hs-CRP, IL-6, and OPG levels were measured. Patients were followed up for cardiovascular events (cardiac death, myocardial infarction, acute coronary syndrome, late revascularization, and nonhemorrhagic stroke).
- RESULTS** Significant CAC (>10 Agatston units) was seen in 236 patients (46.3%); OPG was significantly elevated in patients with increased CAC. In multivariable analyses, OPG retained a strong association with elevated CAC scores after adjustment for age, gender, and other risk factors (odds ratio = 2.84, 95% confidence interval 2.2 to 3.67; $p < 0.01$). Sixteen cardiovascular events occurred during a mean follow-up of 18 ± 5 months. The waist-to-hip ratio, United Kingdom Prospective Diabetes Study (UKPDS) risk score, OPG level, and CAC score were significant predictors of time to cardiovascular events in a univariate Cox proportional hazards model. In the multivariate model, the CAC score was the only independent predictor of adverse events. Levels of hs-CRP and IL-6 were related to neither the extent of CAC nor short-term events.
- CONCLUSIONS** A high proportion of asymptomatic diabetic patients have significant subclinical atherosclerosis. Of the biomarkers studied, only OPG predicted both subclinical disease and near-term cardiovascular events. Therefore, measurement of OPG merits further investigation as a simple test for identifying high-risk type 2 diabetic patients. (J Am Coll Cardiol 2006;47:1850-7)
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Arterial calcification is a prominent feature of atherosclerosis that can be readily quantified noninvasively by radiographic imaging techniques such as electron beam computed tomography (EBCT) (1). Clinical studies have reproducibly shown that high amounts of coronary artery calcification (CAC) predict an increased risk of myocardial infarction and sudden coronary death (2). In patients with diabetes, arterial calcification can occur either as a component of atheroma (intimal calcification) or in the absence of atherosclerotic plaque (medial calcification or Monckeberg sclerosis) (3). Medial calcification is related to the severity

and/or duration of diabetes and typically affects arteries that are less prone to atherosclerosis (4). Although some reports have suggested that it may be associated with an increased risk of cardiovascular complications (5), this is not a consistent finding (6). Notably, medial calcification is unusual in the coronary arteries, implying that when calcification is observed in the coronary arteries, it is almost certainly associated with intimal plaque.

It has been shown that calcification in intimal atherosclerotic lesions is not merely a passive consequence of chronic vascular inflammation but an active process that may lead to a positive feedback loop of calcification and inflammation, driving atherosclerotic disease progression further (7). Contemporary studies have shown similarities between vascular and skeletal calcification suggesting a regulatory role for osteogenic and calcitropic factors (8) in the development of cardiovascular disease. Very recently, osteoprotegerin (OPG), a key factor in bone remodeling, a member of the tumor necrosis factor receptor superfamily (9), and a decoy

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Abbreviations and Acronyms

AU	=	Agatston units
CAC	=	coronary artery calcification
CAD	=	coronary artery disease
EBCT	=	electron beam computed tomography
hs-CRP	=	high-sensitivity C-reactive protein
IL	=	interleukin
IQR	=	interquartile range
OPG	=	osteoprotegerin
OR	=	odds ratio
UKPDS	=	United Kingdom Prospective Diabetes Study

receptor for the receptor activator of nuclear factor- κ B ligand (RANKL), was implicated in human atherogenesis (10). Clinical studies have shown that elevated OPG levels are associated with the progression of vascular calcification in patients on long-term hemodialysis (11), growth of human abdominal aortic aneurysms (12), the presence and severity of coronary artery disease (CAD) (13), and cardiovascular risk in the general population (14).

Because type 2 diabetes increases the risk of coronary atherosclerosis and cardiovascular events, the objectives of our study were: 1) to prospectively evaluate the relationship between plasma OPG levels, the extent of coronary artery calcification (measured by EBCT), and short-term cardiovascular events, and 2) to determine the relationship between OPG and established cardiovascular risk factors, biomarkers of vascular inflammation (high sensitivity C-reactive protein [hs-CRP] and interleukin [IL]-6).

METHODS

Patients. Asymptomatic type 2 diabetic patients without prior evidence of cardiovascular disease were prospectively recruited from four diabetes clinics in secondary care (Northwest London). Inclusion criteria were presence of type 2 diabetes of >1 year duration and age at onset \geq 35 years. Exclusion criteria were age <35 years or >65 years, documented CAD, typical angina pectoris, abnormal resting electrocardiogram (e.g., Q waves, left bundle branch block), cerebrovascular or peripheral arterial disease, renal impairment (creatinine >1.4 mg/dl), or serious life-threatening illness. Diabetes was diagnosed according to American Diabetes Association criteria. Participants were considered to have type 2 diabetes if they had no history of ketoacidosis and if they did not start insulin treatment either in the 2 years after diagnosis or before the age of 40 years. The study was approved by the local research ethics committees of participating hospitals. Detailed medical history including the presence of cardiovascular risk factors, duration of diabetes, presence of microvascular complications, treatment history, and the predicted 10-year absolute coronary heart disease risk based on the Framingham risk function and United Kingdom Prospective Diabetes Study (UKPDS) risk engine (15) were recorded at baseline. A physical examination was performed, and the height,

weight, body mass index, waist-to-hip ratio, blood pressure, and a 12-lead electrocardiogram were also obtained.

Biochemical measurements. Blood and urine samples were obtained for laboratory testing (Diabetes Control and Complications Trial [DCCT]-aligned hemoglobin A_{1c}, lipid profile, urea, creatinine, and urine albumin/creatinine ratio). Serum hs-CRP (ICN Pharmaceuticals, Orangeburg, New York), IL-6 (R&D systems Inc., Minneapolis, Minnesota), and plasma OPG levels (Biomedica, Vienna, Austria) (16) were measured by enzyme-linked immunosorbent assay. Intra-assay and inter-assay coefficients of variation for hs-CRP, IL-6, and OPG measurements were 3.3% and 3.7%, 6.4% and 7.8%, and 5% and 7.5%, respectively.

CAC imaging. The CAC imaging was performed using an EBCT scanner (GE Imatron C-150, San Francisco, California) equipped with high-resolution detectors. Forty contiguous 3-mm slices were obtained at 100-ms scan time per slice in a single breath-hold, starting at the carina and proceeding to the level of the diaphragm. Scan time was 100-ms per slice, synchronized to 40% of the R-R interval. The CAC scores were calculated by an experienced investigator blinded to clinical risk factor information on an Aquarius workstation (TeraRecon Inc., San Mateo, California). Scores were classified into five categories, based on cut-offs that have been widely used in the literature: \leq 10 (minimal or insignificant CAC), 11 to 100 (mild CAC), 101 to 400 (moderate CAC), 401 to 1000 (severe CAC), and >1,000 (extensive CAC) (2).

Patient management and follow-up. Attending physicians were informed of study results; management was left to their discretion. Procedures for follow-up were defined a priori. Telephone interviews and review of medical notes were used to determine the occurrence of cardiovascular events (cardiac death, myocardial infarction, acute coronary syndrome [defined by electrocardiographic changes and/or myocardial markers], late coronary revascularization [$>$ 60 days after CAC scan], or nonhemorrhagic stroke). Subjects who underwent coronary revascularization within 60 days after the CAC scan were excluded from survival analysis to eliminate events that were driven by the results of CAC imaging. In cases in which a study participant experienced more than one end point, only the first end point was counted.

Statistical analysis. Statistical analyses were carried out using the Stata V7 statistical package (Statacorp LP, College Station, Texas) or SPSS version 12.0 (SPSS Inc., Chicago, Illinois). Continuous variables were summarized as mean \pm standard deviation or median \pm interquartile range (IQR). Bivariate correlations between OPG levels, established cardiovascular risk factors, and inflammatory markers (hs-CRP and IL-6) were performed using the Pearson correlation for normally distributed variables and the Spearman rank correlation for variables distributed otherwise. The distribution of variables was assessed examining the

Table 1. Patient Characteristics

Variable	Number	Percentage
Male	309	60.6
Ethnicity		
Caucasian	110	21.6
South Asian	273	53.5
Black	115	22.5
Other (Chinese/Middle Eastern)	12	2.4
Blood pressure >140/90 mm Hg or antihypertensive medication	380	74.5
Treated with blood pressure <140/90 mm Hg	104	20.4
Treated with blood pressure >140/90 mm Hg	140	27.4
Untreated (blood pressure >140/90 mm Hg)	136	26.7
Hyperlipidemia or lipid-lowering medication	318	62.4
Treated with total cholesterol <200 mg/dl	133	26.1
Treated with total cholesterol >200 mg/dl	64	12.6
Untreated (total cholesterol >200 mg/dl)	121	23.7
Family history of premature CAD	158	31
Smoking		
Past	110	21.6
Current	97	19
Insulin only	57	11.2
Insulin + oral agent	51	10
Oral agent	434	85.1
Diet only	19	3.7
Microalbuminuria	74	14.5
Proteinuria	40	7.8
Peripheral neuropathy	90	17.6
Retinopathy	116	22.7

	Mean	SD
Age (yrs)	52.7	8.4
Duration of diabetes (yrs)	8	6
BMI	28.54	5.04
Waist-to-hip ratio	0.94	0.08
CAC	6.2*	0-115.8*; 0-5725†
DCCT-aligned hemoglobin A1c (%)	8.2	1.7
Total cholesterol (mg/dl)	187	36
LDL cholesterol (mg/dl)	107	31
HDL cholesterol (mg/dl)	50	16
Triglycerides (mg/dl)	167	104
hs-CRP (mg/l)	4.28*	1.72-8.01*
IL-6 (pg/ml)	1.93*	1.2-3.1*
Osteoprotegerin (pmol/l)	6.82*	4.68-11.55*

*Median values and interquartile ranges of CAC score, hs-CRP, IL-6, and osteoprotegerin. †The overall range of CAC score.

BMI = body mass index; CAC = coronary artery calcification; CAD = coronary artery disease; DCCT = Diabetes Control and Complications Trial; HDL = high-density lipoprotein; hs-CRP = high-sensitivity C-reactive protein; IL = interleukin; LDL = low-density lipoprotein.

frequency histograms and the normal plots, and by using the Shapiro-Wilk test. Log transformation was applied to normalize the distribution of biomarker values. Ordered logistic regression was performed to identify clinical predictors of coronary calcification and evaluate the association between OPG and CAC. A backward selection procedure was applied to identify candidate variables for the multivariate model. Candidate variables included, from the pool of historical variables, risk factors, and laboratory variables, those univariate predictors with $p \leq 0.2$. For the multivariate model, only those variables with a $p < 0.05$ were retained for the final variable selection.

A univariate and multivariate Cox proportional hazards model was devised to estimate the time to cardiovascular events. From the univariate Cox models, survival curves were calculated based on CAC scores and OPG levels. For this analysis, CAC scores were classified into four categories, namely ≤ 100 , 101 to 400, 401 to 1,000, and $>1,000$ Agatston units (AU), and biochemical marker values (hs-CRP, IL-6, and OPG) were classified into tertiles. Additionally, a receiver operating characteristics curve was plotted for plasma OPG, CAC, and UKPDS and Framingham risk scores with the end point of cardiovascular events, thus evaluating the ability of each variable to classify the primary end point of cardiovascular outcomes. The area under the curve and 95% confidence intervals were calculated for this receiver operating characteristics curve.

RESULTS

The first 510 consecutive patients who fulfilled the eligibility criteria were enrolled (mean age 53 ± 8 years, 61% male). Patient characteristics and biochemical variables are shown in Table 1.

Distribution of CAC. A total of 273 patients (53.5%) had minimal or insignificant CAC (≤ 10 AU). The remaining 236 patients (46.3%) had varying degrees of coronary calcification. The CAC was mild (11 to 100) in 100 patients (19.6%), moderate (101 to 400) in 77 (15.1%), severe (401 to 1,000) in 31 (6.1%), and extensive ($>1,000$) in 28 (5.5%). Image quality was poor in one patient because of significant respiratory motion artifacts.

Ethnic variation in CAC. There was a high proportion of South Asians compared with white Caucasians and black African/Caribbeans (Table 1) because of the geographic location of the study population and the high prevalence of

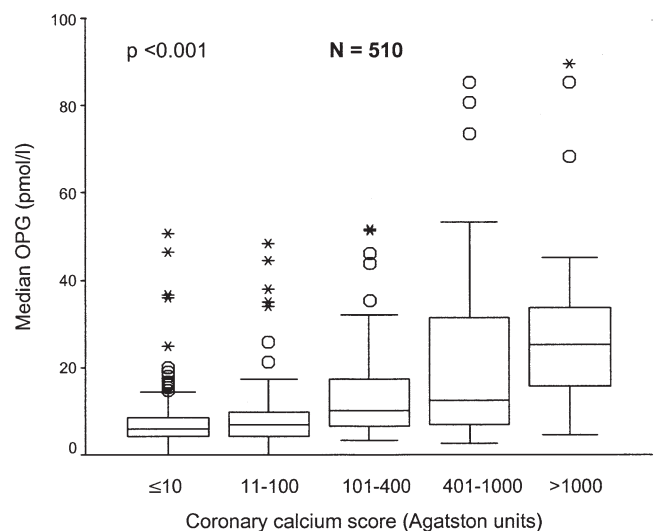


Figure 1. Box and whisker plots illustrate the median and interquartile ranges of osteoprotegerin (OPG) in patients with minimal, mild, moderate, severe, and extensive coronary calcification respectively. **Open circles** show outliers (values between 1.5 and 3 box lengths from the upper edge of the box) and **asterisks** show extreme outliers (values >3 box lengths from the upper edge of the box).

Table 2. Univariate Predictors of Increased Coronary Calcification: Ordered Logistic Regression Analysis

Variable	Odds Ratio (95% Confidence Interval)	p Value
Age*	2.18 (1.76-2.69)	<0.001
Gender		
Female	1	
Male	2.64 (1.88-3.71)	<0.001
BMI	0.96 (0.70-1.32)	0.80
Waist-to-hip ratio†	1.67 (1.34-2.07)	<0.001
Systolic blood pressure	1.20 (1.09-1.32)	<0.001
Diastolic blood pressure	1.18 (1.04-1.35)	0.01
Hyperlipidemia	1.71 (1.21-2.41)	0.002
Family history of premature CAD	1.19 (0.84-1.67)	0.33
Smoking (pack-yrs)		
1-10 yrs	1.23 (0.71-1.82)	
>10 yrs	2.23 (1.45-3.44)	0.001
Duration of diabetes*	1.79 (1.38-2.32)	<0.001
Microalbuminuria	1.04 (0.67-1.62)	0.84
Retinopathy	1.47 (1.01-2.15)	0.04
Peripheral neuropathy	1.43 (0.95-2.15)	0.08
Hemoglobin A1c	1.00 (0.91-1.10)	0.96
hs-CRP (log scale)	0.95 (0.83-1.09)	0.44
IL-6 (log scale)	1.02 (0.98-1.06)	0.45
Osteoprotegerin (log scale)	3.08 (2.42-3.92)	<0.001
Statin therapy	1.72 (1.25-2.38)	0.001
Framingham risk score*	1.2 (0.72-1.68)	<0.001
UKPDS risk score*	1.8 (1.35-2.25)	<0.001

*Odds ratios are given for a 10-U increase in the explanatory variable. †Odds ratios are given for a 0.1-U increase in the explanatory variable.

UKPDS = United Kingdom Prospective Diabetes Study; other abbreviations as in Table 1.

type 2 diabetes in South Asians. Median (interquartile range) CAC scores in black African/Caribbeans, South Asians, and Caucasians were 0 (0 to 42.5) AU, 7.2 (0 to 124.3) AU, and 18.8 (0 to 268.4) AU, respectively ($p < 0.001$).

Relationship between OPG and CAC scores. The median level of OPG was 6.82 pmol/l (IQR 4.68 to 11.55). The OPG levels were significantly elevated in patients with increased CAC (unadjusted odds ratio [OR] = 3.08, 95%

confidence interval [CI] 2.42 to 3.92; $p < 0.001$) (Fig. 1). Univariate predictors of CAC are listed in Table 2. In multivariable analyses, OPG levels retained a strong association with elevated CAC scores after stepwise adjustment for age, gender, and other variables such as hypertension, ethnicity, duration of diabetes, and statin use (OR [age- and gender-adjusted] 2.96 [95% CI 2.34 to 3.78], $p < 0.01$; OR [multivariate adjustment] 2.84 [95% CI 2.2 to 3.67], $p < 0.01$). In contrast, neither hs-CRP nor IL-6 measurements correlated with coronary atherosclerotic plaque burden ($r = 0.02$, $p =$ not significant for hs-CRP; $r = 0.09$, $p = 0.03$ for IL-6) (Fig. 2).

Association between OPG and cardiovascular risk factors. In agreement with previous studies, OPG levels were positively correlated with age as well as waist-to-hip ratio, systolic blood pressure, duration of diabetes, and Framingham and UKPDS risk scores (Table 3), but not inflammatory biomarkers (hs-CRP, IL-6). Patients with evidence of microvascular disease, i.e., retinopathy, peripheral neuropathy, or microalbuminuria, had higher OPG levels in comparison with those without (median OPG = 7.8 [IQR 5.3 to 13.9] vs. 6.1 [IQR 4.3 to 10.5], $p = 0.05$).

Follow-up. Follow-up was completed in 99.6% of patients (average length of follow up, 18 ± 5 months). A total of 16 cardiovascular events occurred (1 cardiac death, 8 nonfatal myocardial infarctions, 2 acute coronary syndromes, 2 late revascularizations, and 3 nonhemorrhagic strokes). No cardiovascular events occurred in subjects with a CAC <10 AU. The majority of events ($n = 14$) occurred in subjects with severe CAC (>400 AU). The OPG levels were higher in subjects with events than in those without: 32 (IQR 13.4 to 74.5) vs. 6.8 (IQR 4.7 to 11) ($p < 0.0001$).

The waist-to-hip ratio, UKPDS risk score, OPG level, and CAC score were significant predictors of time to cardiovascular events in a univariate Cox proportional hazard model, whereas age and male gender were of borderline significance (Table 4). Inflammatory biomarkers such as

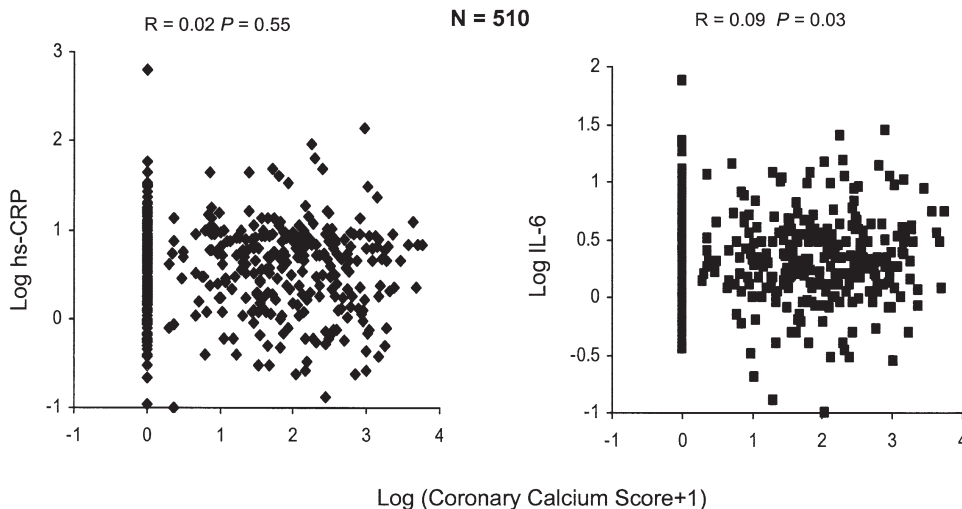


Figure 2. The correlation between markers of inflammation (high-sensitivity C-reactive protein [hs-CRP, in mg/l], interleukin [IL]-6 [in pg/ml]) and the coronary calcium (CAC) score. Log transformation was applied to normalize the distribution of hs-CRP and IL-6 and to reduce the skew of CAC scores.

Table 3. Bivariate Correlation Between Plasma Osteoprotegerin Levels and Cardiovascular Risk Factors

Variable	Correlation Coefficient	p Value
Age	0.30*	<0.0001
BMI	-0.04*	0.35
Waist-to-hip ratio	0.11*	0.01
Systolic blood pressure	0.11*	0.01
Diastolic blood pressure	0.03*	0.57
Smoking (pack-years)	0.07†	0.11
Total cholesterol	0.02*	0.70
LDL cholesterol	0.01*	0.93
HDL cholesterol	0.06*	0.23
Triglycerides	-0.06*	0.17
Hemoglobin A1c	0.01*	0.94
Log hs-CRP	0.01*	0.81
Log IL-6	0.01*	0.87
Duration of diabetes	0.20*	<0.0001
Framingham risk score	0.16†	0.0002
UKPDS risk score	0.26†	<0.0001

*Pearson product moment correlation coefficient is calculated for normally distributed variables. †Spearman rank correlation coefficient is calculated for variables distributed otherwise.

Abbreviations as in Table 2.

hs-CRP and IL-6 were not predictive of short-term cardiovascular events. Compared with patients in the lowest OPG tertile (<5.44 pmol/l), the relative risk of a cardiac event was 5.76 (p = 0.01) for patients with OPG levels in the highest tertile (>9.52 pmol/l) (Fig. 3). In the multivariate model, the CAC score was the only independent predictor of adverse cardiovascular events (Fig. 3).

Receiver-operating characteristic curve analysis (Fig. 4) showed that the CAC score and plasma OPG level were better predictors of short-term cardiovascular risk in comparison to the clinically derived UKPDS/Framingham risk scores (area under the curve values were 0.93, 0.8, 0.75, and 0.62 for CAC, OPG, UKPDS, and Framingham risk scores, respectively [p < 0.0001]). The sum of sensitivity and specificity for prediction of short-term cardiovascular events was maximal at an OPG level of ≥19 pmol/l (sensitivity = 74% [95% CI 48% to 92%], specificity = 91% [95% CI 87% to 93%]).

DISCUSSION

Key findings. In this prospective study involving patients with uncomplicated type 2 diabetes, we evaluated the relationship between established risk factors, biochemical markers (hs-CRP, IL-6, and OPG), and coronary calcification, together with their ability to predict cardiovascular events. Key findings were that OPG is correlated with cardiovascular risk factors (age, waist-to-hip ratio, and systolic blood pressure), duration of diabetes, microvascular disease (retinopathy and peripheral neuropathy), UKPDS/Framingham risk scores, and extent of calcified coronary plaque. Increasing CAC scores and OPG levels were associated with worsening event-free survival. In contrast, biomarkers of inflammation (hs-CRP and IL-6) predicted neither CAC nor near-term cardiovascular events.

OPG and cardiovascular disease. These findings are consistent with those from reports showing a close correlation between OPG and several major cardiovascular risk factors. Plasma levels of OPG increase with age in men and women (16-18). In a study of 286 healthy Korean women age 37 to 73 years (19), OPG was correlated with age, waist-to-hip ratio, and total and low-density lipoprotein cholesterol and follicle-stimulating hormone levels. In another study of 490 Caucasian women age >65 years, OPG was shown to be 30% higher in diabetic compared with nondiabetic subjects (20).

Circulating levels of OPG have also been correlated with surrogate measures of cardiovascular risk including: 1) carotid intima-media thickness in healthy men and women (14), 2) left ventricular hypertrophy (21), and 3) microvascular disease in diabetic subjects (22). Three cross-sectional studies have shown a relationship between OPG levels and the severity of coronary atherosclerosis in symptomatic CAD patients undergoing coronary angiography (13,21,23). More recently, a small case-controlled study (n

Table 4. Univariate Predictors of Cardiovascular Event-Free Survival, Cox Proportional Hazards Model

Variable	Relative Risk (95% Confidence Interval)	p Value
Age*	1.89 (0.92-3.88)	0.08
Gender		
Female	1	
Male	4.30 (0.97-19.1)	0.06
Waist-to-hip ratio†	2.25 (1.23-4.13)	0.009
Hyperlipidemia	0.99 (0.34-2.91)	0.99
Family history of premature CAD	0.77 (0.25-2.43)	0.66
Smoking (pack-yrs)		
1-10 yrs	1.03 (0.32-3.29)	
>10 yrs	0.33 (0.04-2.61)	0.56
Duration of diabetes*	1.66 (0.87-3.17)	0.13
Microalbuminuria	0.86 (0.20-3.83)	0.85
Retinopathy	2.12 (0.76-5.96)	0.15
Peripheral neuropathy	1.53 (0.49-4.82)	0.47
Hemoglobin A1c	0.98 (0.72-1.34)	0.90
Framingham risk score*	1.39 (0.83-2.32)	0.21
UKPDS risk score*	1.43 (1.12-1.82)	0.004
Osteoprotegerin		
<5.44	1	
5.45-9.51	1.03 (0.14-7.34)	
>9.52	5.76 (1.28-26.0)	0.01
hs-CRP		
<2.32	1	
2.35-6.62	0.28 (0.06-1.34)	
>6.62	0.87 (0.29-2.59)	0.27
IL-6		
<1.4	1	
1.4-2.7	1.09 (0.35-3.37)	
>2.7	0.55 (0.14-2.22)	0.61
Calcium score		
≤100	1	
101-400	9.67 (0.88-105)	0.064
401-1,000	69.22 (8.08-585)	<0.0001
>1,000	115.37 (14.17-936)	<0.0001

*Relative risk is given for a 10-U increase in the explanatory variable. †Relative risk is given for a 0.1-U increase in the explanatory variable.

Abbreviations as in Table 2.

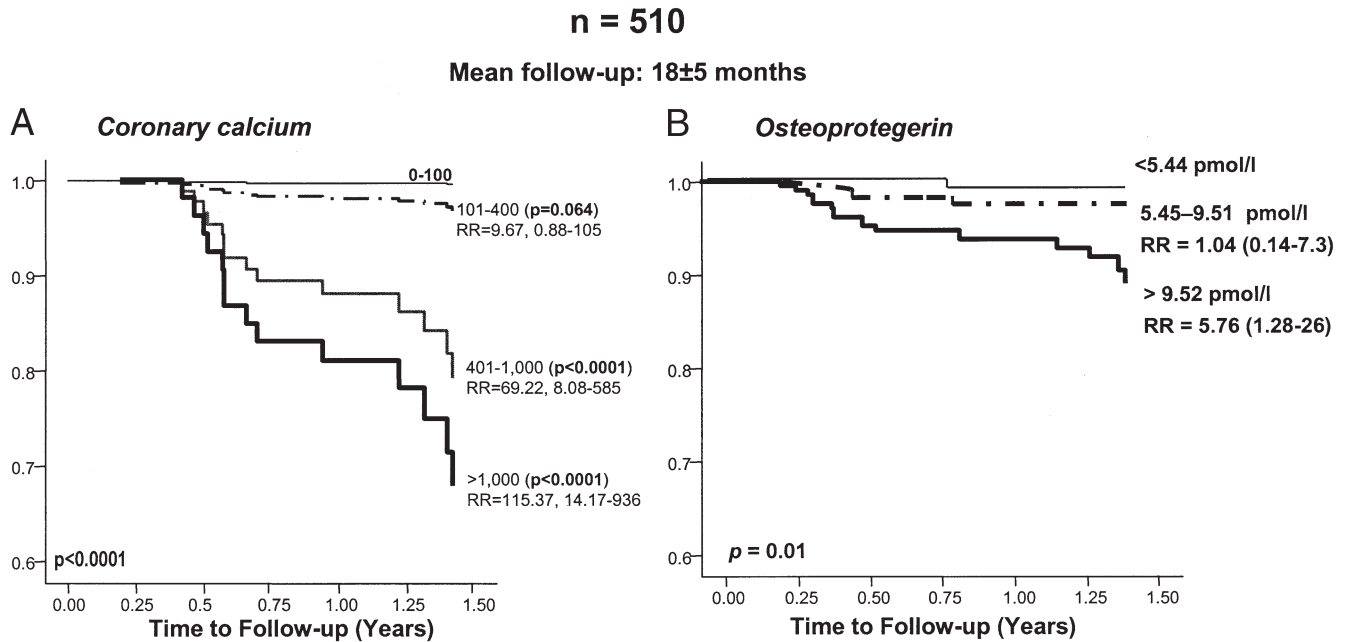


Figure 3. Event-free survival at 18 ± 5 months by the Cox proportional hazards model according to the extent of coronary calcification level (A) and plasma osteoprotegerin (OPG) level (B), respectively. RR = relative risk ratio.

= 40) has confirmed that OPG is an independent predictor of silent CAD in asymptomatic diabetic subjects (24). Recent reports have also established the prognostic value of OPG measurements in healthy men and women (14), and in patients with heart failure after acute myocardial infarction (25).

Our report is the first to show that OPG predicts the extent of CAC (a surrogate measure of total atherosclerotic plaque burden) in asymptomatic diabetic patients. Preliminary data from the population based Multi-Ethnic Study of Atherosclerosis (MESA) are consistent with our observa-

tions (26). In addition, we have also confirmed the prognostic value of elevated OPG levels in this population.

OPG and vascular calcification. Several key regulators of bone formation and bone structural proteins have recently been detected in atherosclerotic plaques (8). The RANKL/RANK/OPG system is emerging as an important regulator of vascular, immune, and skeletal biology (9,10). Genetically altered mice unable to express functional OPG show both osteoporosis and arterial calcification in the aorta and large arteries (27). Interestingly, the intravenous injection of recombinant OPG protein and the transgenic overexpression of OPG have been shown to reverse osteoporosis and prevent vascular calcification (28). Furthermore, it has been shown that administration of recombinant OPG to rats is able to inhibit arterial calcification induced by warfarin and vitamin D (29). Data from experimental studies indicate that pro-inflammatory cytokines such as tumor necrosis factor- α and IL-6 induce OPG expression in smooth muscle and endothelial cells, and OPG in turn modulates various inflammatory signaling pathways (10,12).

All of the above clinical and experimental data suggest an active role for OPG in vascular pathophysiology, but it is not immediately apparent whether OPG is beneficial or injurious to the vasculature. When elevated in patients with atherosclerosis, OPG levels clearly seem to indicate the overall burden of disease (13,14,21,23). This may be because OPG is protective and counterregulatory, as proposed by Browner, et al. (20) and others (14), or it may simply reflect the level of vascular inflammatory processes that underlie the development and evolution of atherosclerotic lesions in patients with extensive disease (10,12).

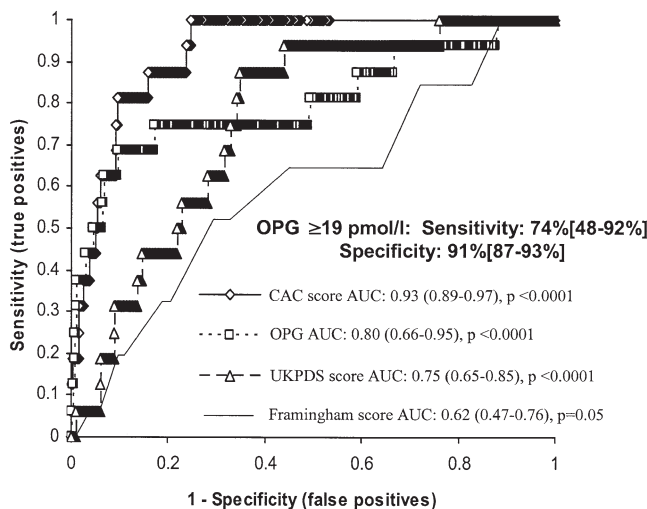


Figure 4. Receiver-operating characteristic curve analysis showing the prognostic value of coronary calcium (CAC) scores, osteoprotegerin (OPG) levels, and United Kingdom Prospective Diabetes Study (UKPDS) and Framingham risk scores in predicting cardiovascular events. The 95% confidence intervals are provided. AUC = area under the curve.

hs-CRP and vascular calcification. Despite large studies showing the relationship between hs-CRP and cardiovascular risk (30), it did not predict near-term cardiovascular events in our study. Consistent with previous reports, however, we found a lack of correlation between CAC scores and hs-CRP (31,32). This may be because they reflect different pathologic processes, i.e., whereas the CAC score is a surrogate marker of the total atherosclerotic plaque burden, hs-CRP seems to provide an assessment of atherosclerotic plaque activity/stability. Overall levels of both hs-CRP and IL-6 were high in this population, and it is possible that inflammation has a permissive role in determining coronary artery plaque volume or stability but that other factors are responsible for driving these processes. Supporting this notion, recent reports have suggested that the predictive value of hs-CRP is relatively modest in comparison with conventional risk factors (33). Because this study was only powered to detect strong risk associations, it does not negate the prognostic value of hs-CRP or IL-6. It merely shows that CAC and plasma OPG are significantly better predictors of short-term CAD risk in this patient group. Our findings are also consistent with data from Schulze et al. (34), who found that hs-CRP levels did not predict cardiovascular events at 18 months in asymptomatic diabetic men, and only became an independent predictor of cardiovascular survival by 5 years.

Study limitations. Attending physicians were informed of CAC results, which may have biased treatment received and assessment of outcome. However, it was not thought ethical to withhold this information given the emerging data on its prognostic power in the general population. Because of the comparatively short follow-up period, both hard and soft end points were included in the analysis. Nevertheless, there were nine hard events, including eight myocardial infarctions.

Conclusions. A high proportion (46.3%) of asymptomatic patients with type 2 diabetes have significant coronary atherosclerosis based on coronary calcium imaging. The extent of CAC was a powerful predictor of cardiovascular event-free survival. Of the biochemical markers studied, only elevated OPG levels predicted subclinical atherosclerosis and near-term cardiovascular events. Since conventional risk factors and inflammatory biomarkers do not provide adequate risk stratification in diabetic subjects, measurement of plasma OPG merits further investigation as a simple test for identifying high-risk individuals.

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