Serum levels of soluble receptor activator for nuclear factor kB ligand play a crucial role in the association of osteoprotegerin with coronary artery disease

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Abstract. Osteoprotegerin (OPG) is a soluble decoy receptor for receptor activator of nuclear factor kB ligand (RANKL), and is implicated in the pathogenesis of atherosclerosis. The aim of the present study was to examine the hypothesis that serum OPG concentrations are increased in patients with stable coronary artery disease (CAD) at different serum levels of soluble RANKL (sRANKL). The study used a case-control design in which consecutively hospitalized individuals were recruited. Fasting blood samples were taken upon admission for serum testing. Participants with previously diagnosed CAD that was asymptomatic or had controlled symptoms constituted the stable CAD group, whereas patients with negative coronary computed tomography angiography results constituted the control non-CAD group. Exclusion criteria included recent acute coronary syndrome, severe heart failure, CAD-complicating autoimmune, blood or thyroid diseases, cancer, elevated temperature with or without infection, severe liver or kidney dysfunction, abnormal calcium metabolism, recent surgery and trauma history. A total of 118 individuals were included in the study. Smoothed plots generated using the recursive method and multivariate models showed that the incidence of stable CAD increased with serum OPG level up to the turning point of 18 pg/ml. This trend was observed at both high [odds ratio (OR), 1.61; 95% confidence interval (CI), 1.04-2.50; P=0.032) and low sRANKL concentrations (OR, 1.52; 95% CI, 1.06-2.17; P=0.022) after adjustment for cardiovascular risk factors. In conclusion, serum OPG levels ≤18 pg/ml are positively associated with stable CAD, regardless of sRANKL levels. In addition, at the same serum OPG level, higher sRANKL levels are associated with a greater incidence of stable CAD compared with lower sRANKL levels. This study identified the relationship between OPG, sRANKL, and stable CAD, and established the reference range for future clinical use.

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Introduction

Early detection of coronary artery disease (CAD) is crucial for the prevention of cardiovascular mortality and morbidity (1). Despite rapid advancements in imaging technology, the clinical diagnosis of CAD may be challenging due to certain patients having conditions that make them unwilling or unable to undergo procedures such as coronary angiography or coronary computed tomography angiography (CTA). These conditions include severe allergies or a history of anaphylactic reaction to contrast medium, bronchial asthma (2), chronic kidney failure (3) or purely psychological reasons with no underlying physical disease. The current American Heart Association/American College of Cardiology guidelines recommend the use of a revised calculator to estimate the 10-year risk of developing a first atherosclerotic cardiovascular disease (ASCVD) event in individuals without prior ASCVD (4). However, for certain patients, the cardiovascular risk factors are not known (5). Thus, alternative biomarkers such as osteoprotegerin (OPG) have been explored.

OPG is a soluble glycoprotein that is a member of the tumor necrosis factor cytokine superfamily (6-8). Receptor activator of nuclear factor κB (RANK) and its ligand (RANKL), the latter of which is also a ligand of OPG, were initially described in the context of bone mass regulation (9). RANKL is produced by osteoblasts and other cell types, such as lymphocytes. The binding of RANKL to RANK on the surface of osteoclast precursors stimulates their differentiation into mature osteoclasts (10). However, OPG acts as a decoy receptor for RANKL, thereby interfering with the binding of RANKL to the RANK cell-surface receptor. This inhibits the formation and activity of osteoclasts, reducing bone

resorption. The balance between RANKL and OPG is critical for the maintenance of bone homeostasis (11).

OPG is also expressed in the vascular system, notably within endothelial and smooth muscle cells. It is a crucial survival factor for endothelial cells, as it is essential for endothelial integrity and function (12). The presence of OPG within atherosclerotic lesions indicates its possible involvement in the pathogenesis of atherosclerosis, which suggests a broader biological significance beyond bone health (13). Numerous human studies have indicated that OPG is an independent predictor of cardiovascular mortality and morbidity, particularly in populations with a high risk of cardiovascular events (14,15). Studies have also demonstrated that elevated serum OPG levels are associated with a higher prevalence of CAD (16,17), suggesting that OPG is a potential predictor of CAD. However, the applicability of these findings to the Chinese population is yet to be determined.

In typical conditions, RANKL is often not present in normal vessels, noncalcified arteries or valves, yet its presence has been identified in atherosclerotic lesions (18). RANKL has been indicated to act as a chemotactic factor by promoting the release of chemokines and stimulating the activity of matrix metalloproteinases (19-21). Atherosclerosis is widely regarded as a chronic inflammatory disease (22). However, population-based studies on the role of soluble RANKL (sRANKL) in CAD have yielded varied results. In some studies, no significant association was found between RANKL concentration and coronary artery calcification or ASCVD (23,24). By contrast, other studies detected a positive or negative correlation between RANKL levels and various forms of cardiovascular disease (25,26). This divergence in findings may be attributed to the interaction between RANKL and OPG, indicating their complex, combined pathogenic influence in the development of atherosclerosis. Further supporting this notion, previous research has suggested that an elevated RANKL:OPG ratio in the circulation could be a valuable biomarker for CAD, potentially aiding in the assessment and prediction of cardiac events (27,28).

The aim of the present study was to evaluate the hypothesis that elevated serum OPG concentrations are associated with the incidence of stable CAD, and that this association is evident for various concentrations of sRANKL. The use of recursive methods for the construction of smooth curves provides an intuitive means for illustrating the relationship between dependent and independent variables (29). In the present study, OPG was used as the independent variable, stable CAD as the dependent variable, and sRANKL as a stratifying factor. This method enabled the plotting of a curve to visually illustrate the curvilinear or linear relationship between OPG levels and stable CAD at various sRANKL concentrations. Successful validation of this hypothesis could be instrumental in the development of novel predictive models for patients with stable CAD.

Patients and methods

Patients. The present case-control study was conducted between June 2021 and December 2021 at the Department of Geriatrics, Union Hospital, Tongji Medical College (Wuhan, China). Fig. 1 shows the full process for the inclusion and exclusion of research participants in the study. Consecutive hospitalized patients during this period who agreed to participate in the study were considered for inclusion, and were divided into two groups: Stable CAD and non-CAD. The non-CAD group comprised patients who received coronary CTA and had no clear coronary atherosclerosis. Participants who were previously diagnosed with CAD and were asymptomatic or had their symptoms under control due to undergoing long-term treatments with various oral medications such as aspirin and statins, were classified into the stable CAD group. The group of patients with CAD included the following two categories: i) Patients with a history of angina, myocardial infarction for >3 months, or coronary intervention histories of >6 months, who had been diagnosed by coronary angiography; and ii) individuals with suspected CAD who underwent diagnostic coronary angiography, and had results consistent with the diagnostic standard of CAD, with the narrowing of at least one major coronary artery by >50% as indicated by coronary angiography (30). Participants with the following conditions were excluded from the study: Myocardial infarction or unstable angina within the previous 3 months, or coronary interventions within the last 6 months; severe heart failure; CAD-complicating autoimmune diseases, blood diseases or cancer; temperature >38˚C with or without a severe infection; severe liver or kidney dysfunction requiring dialysis; thyroid disease; abnormal calcium metabolism; surgery or trauma history within the previous 3 months. The study was approved by the Ethics Committee of Tongji Medical School, Huazhong University of Science and Technology (Wuhan, China; reference no. 2021/0569), and written informed consent was obtained from all participants.

Baseline data. Demographic data and lifestyle characteristics, including age, sex, smoking status and alcohol consumption, were assessed using structured questionnaires. The height and weight of the participants were measured and used to calculate the body mass index (BMI). Serum levels of fasting plasma glucose (FPG), low-density lipoprotein-cholesterol (LDL-C), alkaline phosphatase (ALP), inorganic phosphorus (P) and calcium were examined using an automated biochemical analysis system (AU5800; Beckman Coulter Inc.). The presence of complicating diseases, including hypertension, diabetes, osteoporosis, lacunar infarcts, peripheral arteriosclerosis and chronic kidney disease, was also recorded in the baseline data.

Enzyme-linked immunosorbent assays (ELISAs). Blood specimens were collected from the participants in the morning after an overnight fast, and the serum samples were stored at -70˚C. The double-antibody sandwich ELISA method was used to determine the serum concentrations of OPG and sRANKL. Commercially available kits for OPG (cat. no. H286; Nanjing Jiancheng Bioengineering Institute) and sRANKL (cat. no. H284; Nanjing Jiancheng Bioengineering Institute) were used. Briefly, anti-human OPG or RANKL monoclonal antibodies were coated on the microplate. The patient samples and standards were applied, the OPG or RANKL present in the samples bound to the antibodies fixed on the plate, and the unbound components were then washed away. Biotin-labeled anti-human OPG or RANKL polyclonal antibodies were subsequently added, which bound to the OPG or RANKL already

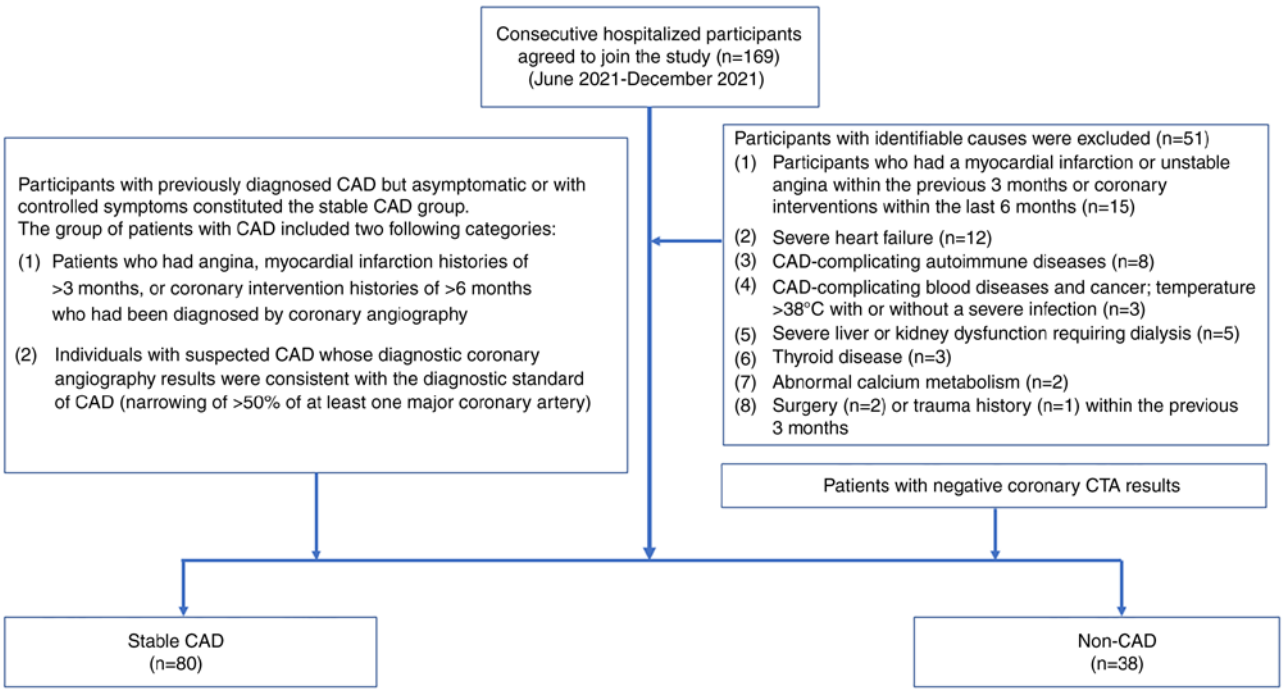

Figure 1. Flowchart of the selection of participants. CAD, coronary artery disease; CTA, computed tomographic angiography.

attached to the microplate, and the unbound components were washed away. Next, streptavidin-labeled horseradish peroxidase, which specifically recognizes biotin, was added to form a complex. After washing away the unbound components, a chromogenic substrate solution was added, which gradually turned the solution blue. The addition of stop solution changed the color to yellow and halted the reaction. The absorbance was measured using an ELISA reader at a wavelength of 450 nm (Thermo Fisher Scientific, Inc.). To determine the concentration of each sample, a standard curve was drawn based on results obtained using the standard solution. The results were expressed in pg/ml. All samples were measured in duplicate, and the results were averaged.

Statistical analysis. Normally distributed variables are presented as the mean ± standard deviation, while abnormally distributed variables are presented as the median and quartiles. Qualitative data are expressed as the number with the percentage in parentheses. Comparisons between groups were made using unpaired Student's t-test for normally distributed parameters, or Kruskal-Wallis test for variables with a skewed distribution. For categorical variables, the χ^2 test was used to analyze differences between the non-CAD and CAD groups, or Fisher's exact test was employed for categorical variables with expected frequencies <5. Spearman's correlation analysis was used to analyze the correlation between two continuous variables. Pearson's χ^2 test was used to test the correlation between dichotomous variables. Subsequently, a univariate analysis model was utilized to examine the association of OPG and other risk factors with the presence of CAD. The consistency of these associations was then explored in various subgroups (stratified analyses). Globally, individuals aged 65 and above are categorized as elderly (31), with age groups typically classified as under 65 and 65 and older. Smooth curve fitting analysis revealed a biphasic relationship between FPG and CAD, characterized by an initial decrease followed by an increase. This analysis identified a nadir at 4.7 mmol/l, leading to the stratification of FPG levels into two groups: <4.7 and ≥4.7 mmol/l. Similarly, BMI exhibited a similar biphasic curve in relation to CAD, with a minimum value of 28.96 kg/m^2. Consequently, BMI values were categorized into <28.96 kg/m^2 and ≥28.96 kg/m^2 groups, reflecting distinct risk profiles for CAD. ALP, LDL-C, calcium and P were categorized into tertiles. The association between serum OPG concentration and CAD in individuals in different sRANKL-level groups was analyzed, with adjustment for potential confounding factors through smooth curve fitting by the recursive method. The potential confounders were determined based on covariate screening criteria, which included effect factors producing a >10% change when introducing or eliminating covariates in the basic or regression models. Finally, a multivariate piecewise linear regression model was conducted to evaluate the independent relationship between OPG and the presence of CAD in the total sample and in various sRANKL-level groups based on smooth curve fitting.

Results

Clinical and baseline characteristics of the subjects. Table I presents the clinical and baseline data of the study participants. A total of 118 patients were enrolled. The patients with CAD were older than those without CAD and had a higher likelihood of having a history of diabetes mellitus and peripheral arteriosclerosis (P<0.001). The patients with CAD also exhibited significantly higher serum levels of OPG and sRANKL compared with the non-CAD controls [OPG, median (interquartile range), CAD, 8.44 (5.10-14.87) vs. non-CAD, 7.00 (4.49-9.63) pg/ml, P=0.005; sRANKL, median

Table I. Demographic characteristics and biochemical data of the subjects.

Variables	Non-CAD (n=38)	Stable CAD (n=80)	P-value[a]
Demographic characteristics			
Age, mean ± SD, years	55.9±13.5	69.4±12.4	<0.001
Male, n (%)	29 (76.32)	65 (81.25)	0.534
Body mass index, mean ± SD, kg/m^2	26.22±2.87	26.65±3.37	0.495
Current smoker, n (%)	18 (47.37)	30 (37.50)	0.308
Current drinker, n (%)	9 (23.68)	17 (21.25)	0.766
Complicating disease history, n (%)			
Hypertension	30 (78.95)	70 (87.50)	0.227
Diabetes mellitus	0 (0.00)	35 (43.75)	<0.001
Lacunar infarcts	16 (42.11)	49 (61.25)	0.051
Peripheral arteriosclerosis	12 (31.58)	68 (85.00)	<0.001
Osteoporosis	4 (10.53)	21 (26.25)	0.051
Chronic kidney disease	2 (5.26)	10 (12.50)	0.224
Laboratory test results			
Fasting plasma glucose, mean ± SD, mmol/l	4.93±0.70	5.33±1.46	0.114
Alkaline phosphatase, mean ± SD, U/l	72.03±21.24	74.29±20.32	0.579
LDL-C, mean ± SD, mmol/l	2.52±0.71	2.25±0.77	0.072
Total calcium, mean ± SD, mmol/l	2.24±0.11	2.26±0.12	0.304
Phosphorus, mean ± SD, mmol/l	1.15±0.17	1.16±0.17	0.901
OPG, median (IQR), pg/ml	7.00 (4.49-9.63)	8.44 (5.10-14.87)	0.005
sRANKL, median (IQR), pg/ml	17.09 (8.66-39.01)	31.65 (12.60-101.62)	<0.001

[a]Comparisons between groups for continuous data were made using unpaired t-tests for normally distributed variables and Kruskal-Wallis tests for variables with a skewed distribution. χ^2 test was used to analyze the differences between the non-CAD and stable CAD groups for categorical variables. Fisher's exact test was performed for the analysis of categorical variables with expected counts <5. CAD, coronary artery disease; n, number; LDL-C, low density lipoprotein-cholesterol; OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor-κB ligand; IQR, interquartile range.

(interquartile range), CAD, 31.65 (12.60-101.62) vs. non-CAD, 17.09 (8.66-39.01) pg/ml, P<0.001].

Crude associations between serum OPG levels and the presence of CAD in patient subgroups. Associations between different variables and CAD were examined in the total study population by univariate analysis using logistic regression analysis with CAD as the dependent variable. Initially, a positive association was found between serum OPG levels and the presence of CAD [odds ratio (OR), 1.11; 95% confidence interval (CI), 1.03-1.19; P=0.009], as depicted in Table SI. In addition, significant positive associations were found for CAD with age, peripheral arteriosclerosis, sRANKL and LDL-C (P<0.05). These associations were further investigated through stratified analyses, which consistently revealed a positive association between OPG and CAD across various subgroups, as illustrated in Fig. 2. Unfortunately, the model was not able to predict the association of CAD with diabetes mellitus, as all the diabetic patients included in the study had been diagnosed with CAD.

Correlation between serum OPG level and the presence of CAD. The adjusted smoothed plots in Fig. 3 suggest a nonlinear relationship between serum OPG and the presence of CAD after adjustment for confounding variables. Specifically, the presence of CAD increased as serum OPG levels increased up to the turning point at 18 pg/ml, as shown by the curve exhibiting one breakpoint and a two-stage change. For serum OPG levels >18 pg/ml, the estimated dose-response curve was consistent with a horizontal line. We further divided OPG <18 pg/ml into two equal groups (<9 and 9-18 pg/ml) in order to obtain the OR value by generalized linear regression. Furthermore, in analyses adjusted for age and sex, individuals with middle levels of OPG (9-18 pg/ml) had an OR for CAD of 2.46 (95% CI, 0.82-7.39; P=0.110) compared with those with low levels of OPG (<9 pg/ml), as shown in Table II. However, following progressive adjustment for various cardiovascular risk factors, namely smoking, drinking, peripheral arteriosclerosis, osteoporosis, FPG, LDL-C, and hypertension, the patients with middle levels of OPG had an OR for CAD of 6.66 compared with those with low levels of OPG (95% CI, 1.35-32.79; P=0.020, P-value for trend=0.013; Table II). No significant association was found between serum OPG levels and increasing age or other risk factors.

Association between serum OPG level and the presence of CAD in individuals with low and high serum sRANKL levels. To investigate the association between serum OPG and the presence of CAD, sRANKL levels were divided into low and high concentrations. Specifically, the participants were split

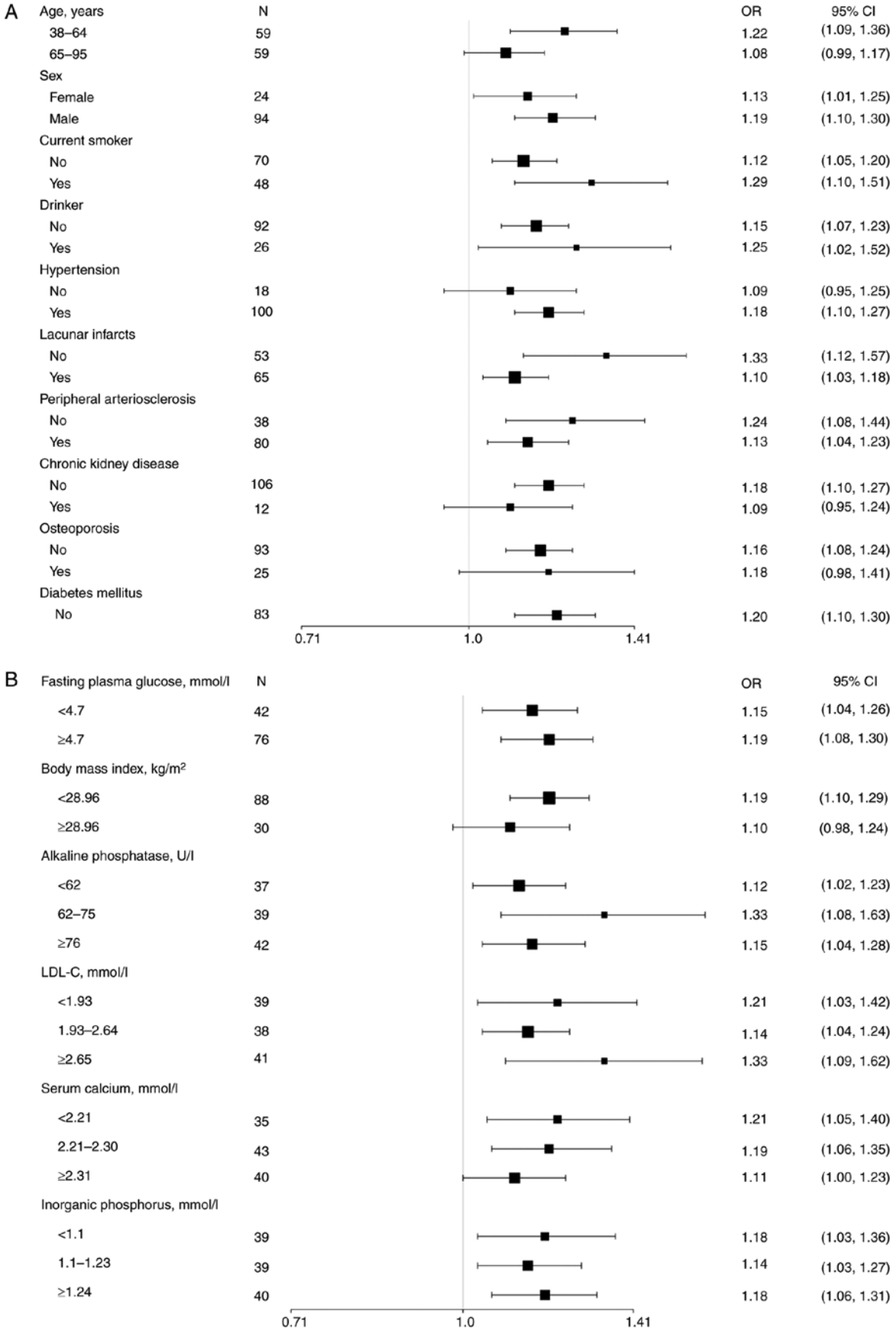

Figure 2. Univariate analysis models were used to examine the relationship between OPG and the presence of CAD in different subgroups using stratified analyses. (A) Stratification was conducted based on the basic characteristics of the patients, including age, sex, current smoking and drinking habits, as well as comorbidities including hypertension, lacunar infarcts, peripheral arteriosclerosis, chronic kidney disease and osteoporosis. The model of association between OPG and CAD in individuals with diabetes mellitus was unsuccessful, as all patients in this subgroup had already been diagnosed with CAD. (B) Stratification was performed according to body mass index or various laboratory results associated with CAD or its comorbidities. Data are expressed as OR (95% CI). OR, odds ratio; CI, confidence interval; OPG, osteoprotegerin; CAD, coronary artery disease; LDL-C, low-density lipoprotein-cholesterol.

Table II. Threshold effect analysis of the association between OPG and CAD using piecewise linear regression.

	Crude model		Model 1		Model 2	
Variable	OR (95% CI)	P-value	OR (95% CI),	P-value	OR (95% CI),	P-value
OPG ≤18 pg/ml	1.05 (0.94, 1.17)	0.367	1.11 (0.98, 1.26)	0.102	1.32 (1.07, 1.63)	0.011
OPG categories, pg/ml						
<9 (n=69)	Reference		Reference		Reference	
9-18 (n=31)	1.35 (0.55, 3.30)	0.511	2.46 (0.82, 7.39)	0.11	6.66 (1.35, 32.79)	0.02
P-value for trend		0.511		0.11		0.013

OPG is considered as a continuous and as a categorical variable. When considered as continuous, OPG levels ≤18 pg/ml display a positive association with the presence of CAD. When considered as a categorical variable, middle levels (9-18 pg/ml) of OPG are associated with a greater proportion of CAD compared with lower levels (<9 pg/ml). Covariates included in the piecewise linear regression models were as follows: Crude model, unadjusted; Model 1, adjustment for age (smooth) and sex; Model 2, adjustment for the same variables as in Model 1 plus smoking, drinking, peripheral arteriosclerosis, osteoporosis, fasting plasma glucose, low-density lipoprotein-cholesterol and hypertension. OR, odds ratio; CI, confidence interval; OPG, osteoprotegerin.

Table III. Association between OPG and the presence of coronary artery disease at low and high sRANKL concentrations analyzed using multivariate regression.

	Model 1		Model 2	
sRANKL concentration, pg/ml	OR (95% CI)	P-value	OR (95% CI)	P-value
8.66-24.69	1.46 (1.03, 2.08)	0.036	1.52 (1.06, 2.17)	0.022
25.2-101.62	1.15 (0.92, 1.43)	0.213	1.61 (1.04, 2.50)	0.032

Covariates included in multivariate regression models for low (8.66-24.69 pg/ml) and high (25.2-101.62 pg/ml) sRANKL concentrations were as follows: Model 1, adjustment for age (smooth) and sex; Model 2, adjustment for the same variables as in Model 1 plus smoking, drinking, peripheral arteriosclerosis, osteoporosis, fasting plasma glucose, low-density lipoprotein-cholesterol, and hypertension. OR, odds ratio; CI, confidence interval; OPG, osteoprotegerin; sRANKL, soluble receptor activator of nuclear factor-kB ligand.

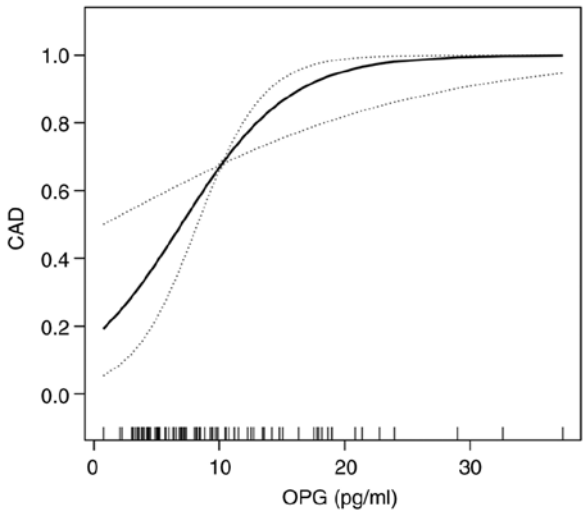

Figure 3. Smoothed plots for the nonlinear relationship between serum OPG and the presence of CAD after adjustment for certain variables. The presence of CAD increased with serum OPG level up to the turning point (OPG=18 pg/ml), as shown by the curve exhibiting one breakpoint and a two-stage change. With serum OPG >18 pg/ml, the estimated dose-response curve was consistent with a horizontal line. Adjustments were made for age (smooth), sex, smoking, drinking, peripheral arteriosclerosis, osteoporosis, fasting plasma glucose, low-density lipoprotein cholesterol and hypertension. OPG, osteoprotegerin; CAD, coronary artery disease.

into two groups, each comprising 59 individuals. Those with sRANKL levels between 8.66 and 24.69 pg/ml were assigned to the low concentration group, while the remaining with sRANKL concentrations ranging from 25.2 to 101.62 pg/ml were classified as the high concentration group. Notably, the adjusted smoothed plots in Fig. 4 indicate that the percentage of individuals with higher sRANKL levels who had CAD was higher compared with that of individuals with lower sRANKL levels, following adjustment for various cardiovascular risk factors. Furthermore, after limiting OPG to ≤18 pg/ml, the results showed that for every one-unit increase in serum OPG, there was a 52% increase in the presence of CAD in the low sRANKL group (OR, 1.52; 95% CI, 1.06-2.17; P=0.022) and a 61% increase in the presence of CAD in the high sRANKL group (OR, 1.61; 95% CI, 1.04-2.50; P=0.032) (Table III).

No correlation was found between OPG and sRANKL using Spearman's correlation analysis (Fig. S1). Spearman's correlation analysis was then applied to further clarify whether serum OPG or sRANKL levels correlated with the CAD risk factors collected in the present study (Figs. S2 and S3). The analysis showed that age was positively correlated with sRANKL (P<0.0001; Fig. S3A), but not with OPG (Fig. S2A). No significant correlation of OPG or sRANKL with other continuous variables including FBG, LDL-C, BMI, ALP, serum calcium

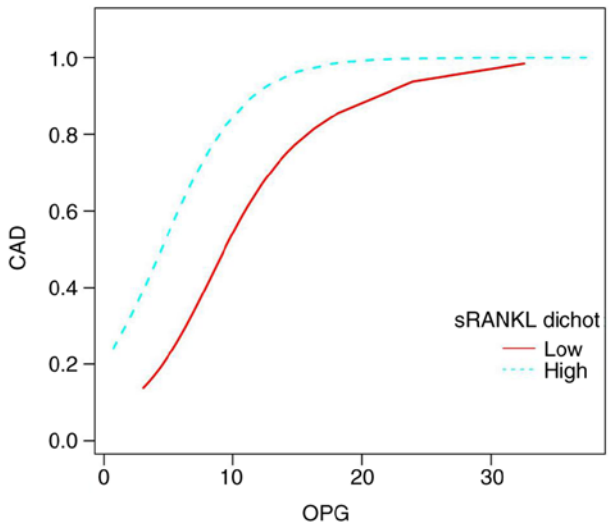

Figure 4. Smoothed plots for the correlation between serum OPG and the presence of CAD in patients with low and high sRANKL levels. The low sRANKL group had levels of 8.66-24.69 pg/ml (n=59), while the high sRANKL group had levels of 25.2-101.62 pg/ml (n=59). Serum OPG levels <18 pg/ml were positively associated with stable CAD, regardless of sRANKL levels. Additionally, higher sRANKL levels were positively associated with a greater presence of stable CAD compared with lower sRANKL levels, at the same serum OPG levels, when adjustment for age (smooth), sex, smoking, drinking, peripheral arteriosclerosis, osteoporosis, fasting plasma glucose, low-density lipoprotein cholesterol and hypertension. CAD, coronary artery disease; OPG, osteoprotegerin. sRANKL, soluble receptor activator of nuclear factor-kB ligand; dichot, dichotomous.

and P was detected (Figs. S2 and S3). Pearson's χ^2 test showed that serum OPG was associated with peripheral arteriosclerosis (χ^2=0.082, P=0.018) and diabetes mellitus (χ^2=6.447, P=0.040), while sRANKL was associated with lacunar infarction (χ^2=5.789, P=0.016), peripheral arteriosclerosis (χ^2=7.608, P=0.006) and osteoporosis (χ^2=4.111, P=0.043).

Discussion

Using the recursive method, the present study revealed a nonlinear relationship between serum OPG levels and the presence of CAD. A positive association between the presence of CAD and serum OPG was observed when the serum OPG level was ≤18 pg/ml. However, when the serum OPG concentration was >18 pg/ml, the incidence of CAD no longer increased with increasing serum OPG concentration. Additionally, patients with higher sRANKL levels tended to have a higher risk of CAD compared with those with lower sRANKL levels, at the same serum OPG levels.

The present study used the recursive method for curve fitting and demonstrated that OPG and CAD had a curvilinear relationship, in which the turning point of the curve occurred at a serum OPG concentration of 18 pg/ml. The turning point was identified using the threshold selection method derived from curve fitting (32-36). Recursive methods for smooth curve fitting were first applied, followed by segmental modeling to identify any turning points. It was observed that when the serum OPG concentration was >18 pg/ml, the curve became horizontal. Multivariate linear regression analysis yielded a result of 8,028 (0, Inf) with P=0.999, suggesting that at OPG concentrations >18 pg/ml, OPG levels are infinitely close to those associated with CAD. Conversely, when the serum concentration of OPG was ≤18 pg/ml, curve fitting revealed a linear correlation between OPG and CAD. Multivariate linear regression with adjustment for age, sex and various cardiovascular risk factors revealed an OR of 1.32 (95% CI 1.07-1.63; P=0.011), further supporting the hypothesis that elevated OPG is an independent predictive factor for CAD.

The curved relationship displayed between serum OPG levels and the incidence of CAD in the present study suggests that the association between these two factors exhibits a saturation effect. This finding is supported by a study of mice conducted by Morony *et al* (37), which observed similar results. In that study, LDL receptor-knockout mice were fed an atherogenic diet and subsequently treated with recombinant OPG or vehicle for 5 months, and plasma OPG levels were measured from the initiation of the atherogenic diet. The study found a significant increase in plasma OPG levels in the first month after the start of the diet, but no further increase in OPG levels despite the progression of atherosclerosis in the vehicle-treated mice. The results of the present study combined with the findings in mice indicate that OPG may act as a biomarker of CAD within a certain range.

It has been suggested that an increase in serum OPG might occur in response to vascular calcification or atherosclerosis rather than being a cause of these conditions, and may be intended to regulate the disease process (14). We hypothesize that the elevation of OPG could be a protective response to endothelial dysfunction in patients with CAD, as endothelial dysfunction is a common complication in these patients (38). Therefore, the elevation of OPG may be indicative of the cumulative burden of risk factors in patients with CAD. The present study found consistent ORs for the associations between OPG levels and CAD in various subgroups, including those for age, sex, smoking, drinking, hypertension, lacunar infarcts, peripheral arteriosclerosis, chronic kidney disease, osteoporosis, FPG, BMI, ALP, LDL-C levels, serum calcium and P levels. The study indicated that elevated OPG levels increase the burden of CAD independently of the aforementioned traditional risk factors.

OPG acts in combination with one of its ligands, RANKL. However, previous studies of the relationship between RANKL and CAD have not yielded conclusive results (39,40). The present study provides clinical evidence demonstrating that the incidence of CAD among patients with higher levels of sRANKL was elevated compared with that among patients with lower sRANKL levels, even when serum OPG levels were the same. This suggests that OPG acts in conjunction with RANKL in stable CAD. However, no correlation was found between serum OPG and sRANKL concentrations. It may be speculated that the concentrations of OPG and/or sRANKL are associated with the expression of RANK and the quantity of RANKL expressed by cells such as T cells, or that they are influenced by other inflammatory factors within the human body (39,24). The present found that serum sRANK levels were positively associated with age, peripheral arteriosclerosis, lacunar infarction and osteoporosis, further suggesting that sRANKL is an inflammation-related factor. Therefore, we hypothesize that the role of OPG in the neutralization of RANKL activity via inhibition of its binding to RANK can be attributed to three effects. First, the binding of

RANKL to RANK promotes the pathological differentiation of healthy vascular smooth muscle cells (VSMCs) into calcified cells with an osteoblastic phenotype (24,40), and OPG inhibits this calcification. Second, RANKL significantly increases matrix metalloproteinase activity in VSMCs from patients with unstable angina, and OPG counteracts the effect of RANKL by inhibiting its binding to RANK (41,42). Last, the binding of OPG with RANKL may inhibit the rapid clearance of RANKL from the serum, thereby stabilizing its levels and enhancing its actions (41). These studies indicate that OPG and sRANKL antagonize each other. Since OPG contributes to an incomplete compensatory mechanism in atherosclerosis, the present research suggests that RANKL is indispensable in the role of OPG in CAD.

The present study primarily highlights the relationships between OPG, sRANKL and asymptomatic or well-controlled symptomatic CAD. Therefore, the measurement of OPG and sRANKL serum levels in asymptomatic individuals in addition to the analysis of traditional cardiovascular risk factors may serve as an initial screening method for patients with this type of CAD in clinical practice. However, it is important to note that further confirmation of this conclusion is required through large-scale prospective cohort studies.

There were some limitations to the present study. First, as this was a case-control study, the number of patients in the two groups was not exactly matched. The patients were recruited from inpatient wards, and most of them were undergoing regular check-ups due to pre-existing diseases. Although there were also some patients who underwent regular check-ups despite not having CAD, the number of patients with CAD was higher than those without CAD during the study period. This imbalance might have led to an underrepresentation of the non-CAD population, resulting in a situation where a particular group or demographic within a population was not adequately represented, which could potentially impact the findings of the study. Second, the number of patients in the study with diabetes was limited. As a result, the conclusions of the study cannot be applied to the diabetic population. However, it is widely accepted that diabetes mellitus is 'CAD-risk equivalent' (43). Therefore, additional biomarkers may not be necessary for assessing the presence of CAD in diabetic patients, although recent guidelines recommend further CAD risk stratification in patients with type 2 diabetes mellitus (44). Third, although the study did not assess the degree of coronary artery sclerosis in the group of patients with CAD due to them having been diagnosed prior to the study, a relationship between OPG levels and the degree of coronary artery atherosclerosis has previously been reported (16). Therefore, the curve derived in the study is not suitable for establishing if OPG and sRANKL are associated with the degree of coronary artery sclerosis. Additionally, due to the small size of the study population, it was not possible to include another group of patients with CAD to validate the predictive value of OPG for CAD, as recommended by the TRIPOD statement for prediction model studies (45). Finally, it is important to note that the subjects who were physically examined had reasonable control over cardiovascular risk factors such as FPG and lipid levels, indicating a high level of concern for their health. However, the study did not account for potential confounding factors such as medication use.

In conclusion, independently of age, sex, smoking, drinking, lacunar infarcts, peripheral arteriosclerosis, osteoporosis, FBP, hypertension, LDL-C and BMI, a higher prevalence of CAD is associated with serum OPG levels ≤18 pg/ml. Moreover, the incidence of CAD among patients with higher sRANKL levels was higher than that among patients with lower sRANKL levels at the same concentration of OPG. However, further studies are necessary to establish a predictive model for CAD based on OPG and sRANKL levels.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

XF and SG conceived and designed the experiments. All authors performed the experiments. HW and SZ collected data, and XF and MZ analyzed the data. XF, HW and SZ drafted the paper, and BW revised the paper. All authors read and approved the final version of the manuscript. HW, SZ and XF confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Tongji Medical School, Huazhong University of Science and Technology (Wuhan, China; approval no. 2021/0569). Written informed consent was obtained from all subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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