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Biomarkers of the osteoprotegerin pathway: clinical correlates, subclinical disease, incident CVD and mortality

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Abstract

Objective—Experimental evidence identified the osteoprotegerin [OPG]/receptor activator of nuclear factor–kappa-B [RANK]/RANK ligand [RANKL] pathway as a candidate system modulating vascular remodeling and cardiovascular disease (CVD).

Methods and Results—Serum concentrations of OPG and RANKL were measured in 3250 Framingham Study participants (54% women, 61±9 years). During a median follow-up of 4.6 years, 143 (of 3084 free of CVD at baseline) participants developed a first CVD event and 235 died. In multivariable models OPG was associated with increased hazards for incident CVD and mortality (HR: 1.27; 95% CI, 1.04 to 1.54 and HR: 1.25; 95% CI, 1.07 to 1.47 per one-SD increment in log-OPG, respectively). Log-OPG was positively related to multiple CVD risk factors including age, smoking, diabetes, systolic blood pressure and prevalent CVD. In a subsample (n=1264), the prevalence of coronary artery calcification, measured by computed tomography, increased non-significantly with OPG-quartiles. RANKL concentrations displayed inverse associations with multiple CVD risk factors including smoking, diabetes and antihypertensive treatment, and were not related to coronary artery calcification or incident CVD or mortality.

Conclusions—Our prospective data reinforce OPG as marker for CVD risk factor burden and predictor for CVD and mortality in the community.

Keywords

OPG; RANKL; CVD; vascular remodeling; biomarker

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Cardiovascular diseases (CVD) develop over the human life-course and acute CVD events are often preceded by progressive modifications in the vessel wall referred to as vascular remodeling.[1] Understanding the biologic basis of vascular remodeling is, therefore, critical to prevent disease progression and clinical CVD events.

Initially described in the context of bone mass regulation,[2] RANK (receptor activator of nuclear factor kappa-B), RANK ligand (RANKL), and osteoprotegerin represent an interesting putative pathway in vascular remodeling and atherosclerotic disease.3 OPG acts as a decoy receptor for RANKL,3 thereby interfering with RANKL-binding to its cell-surface receptor RANK. The RANK-RANKL interaction triggers vascular permeability,4 cytokine release, monocyte transmigration and monocyte matrix metalloproteinase activity.5 In patients with unstable angina (as compared to stable angina), higher expression of RANKL in T-cells and of monocytic RANK have been reported.[6]

OPG is expressed in the vascular system, including endothelial and smooth muscle cells and is modulated by pro-inflammatory cytokines like interleukin-1 and tumor necrosis factor (TNF) alpha.[5] It also serves as a survival factor for endothelial cells.7 In addition, OPG and RANKL were both found in atherosclerotic lesions.5 Mice with targeted disruption of the *OPG* gene display medial calcification of the aorta and renal arteries[8] and OPG application inhibited Vitamin D-induced and warfarin-induced vascular calcification in rats.[9] Clinical studies have observed higher circulating OPG levels in patients with unstable angina and with acute myocardial infarction, but not in patients with stable angina or established coronary disease suggesting that the OPG/RANK/RANKL system might modulate plaque stability.[3, 6, 10] In addition, OPG was positively associated with the severity of coronary artery disease[11] and predicted heart failure hospitalizations and mortality in patients with acute coronary syndromes.[12] Few moderate sized epidemiological studies have examined the relations of OPG and RANKL to CVD events and mortality in the community.13–15 In the Bruneck study, for example, positive associations of OPG13 and RANKL[14] with incident CVD have been reported. In a prospective case-control study nested within the European Prospective Investigation into Cancer and Nutrition (EPIC-Norfolk) study, baseline OPG levels were associated with incident coronary events, but not baseline RANKL levels.[16]

Given the above mentioned clinical and experimental evidence, we aimed to assess whether circulating OPG and RANKL concentrations were associated with incident CVD events and mortality as well as with key CVD risk factors and coronary calcification, an important intermediate phenotype in the CVD continuum.

Methods

Study sample

Design and recruitment strategies for the Offspring cohort of the Framingham Heart Study have been described elsewhere.[17] Since the initiation of the study in 1971, participants are seen in the Heart Study clinic every 4 to 8 years on average. During each visit, the CVD risk profile is assessed; a physical examination is performed and a medical history is obtained, focusing on CVD symptoms and events since the last exam. Of 3539 participants who attended Offspring examination cycle 7, 289 Offspring cohort participants were excluded for serum creatinine >2 mg/dL (n=11) or missing OPG levels (n=278) at the baseline examination cycle 7. An additional 47 individuals had OPG levels available but missing RANKL values. Individuals with creatinine levels above 2 mg/dL were excluded because impaired renal function could affect circulating biomarker concentrations. After exclusions, 3250 Offspring cohort participants remained eligible for the present analyses involving OPG

and 3203 for the analyses involving RANKL. For the analyses of incident CVD, individuals with prevalent CVD at baseline were excluded. The study protocol was approved by the Institutional Review Board at the Boston University Medical Center and all participants provided written informed consent.

Biomarker measurement

Blood was drawn from fasting participants; usually between 8.00 and 9.00 AM at Offspring examination cycle 7, immediately centrifuged and stored at −80 C until biomarkers were assayed. OPG and RANKL were measured using commercially available second-generation high-sensitivity ELISA assays (manufactured by Biomedica, Vienna, Austria and distributed in the U.S. by ALPCO Diagnostics). The detection limits for OPG and RANKL were 0.14 pmol/L and 0.02 pmol/L, respectively. The inter-assay coefficient of variation for OPG and RANKL were 4% and 33% respectively; and the intra-assay CV for OPG and RANKL were 3.08% and 3.51%, respectively.

Determination of coronary artery calcification

A total of 1422 participants underwent multi-detector computed tomography (MDCT) as described in detail elsewhere.18 Participants from larger families within the Heart Study were preferentially selected for the MDCT substudy to allow the conduct of genetic studies for CT measures. For logistic reasons participants living in the New England area were targeted. Men and women had to be at least 35 and 40 years old, respectively, and weigh less than 150 kg (due to scanner limitations). Pregnancy was also a contraindication.18 Coronary artery calcification (CAC) was defined as a CAC score ≥90% age- and sex-specific percentile in a healthy reference sample within the Framingham Heart Study.18 In 1264 participants of the MDCT substudy, OPG and RANKL levels were available. The average time interval between the clinic exam (when the blood draw for the biomarker analyses was performed) and the date of the CT was 4.2 years (range 1.5 years to 6.5 years).

Definition of events

All participants underwent continuous surveillance for incident CVD events and death. A team of 3 physicians reviews all available information, hospitalization records and physician charts to adjudicate outcome events. Similarly, suspected cerebrovascular events are adjudicated by a panel of 3 investigators. For the different CVD and cerebrovascular outcomes, standardized criteria were used as detailed elsewhere.[19] CVD events included fatal (n=6) and non-fatal MI (n=58), coronary insufficiency (n=6), heart failure (n=40), and stroke (n=33).

Statistical analyses

Given their skewed distributions, OPG and RANKL were both natural logarithmically transformed to improve normality. Correlations between log-biomarkers were obtained using Pearson's correlation coefficient.

Clinical correlates—Clinical correlates of serum log-OPG and log-RANKL were determined using forward selection regression models ($p<0.05$ for model entry). Eligible covariates for these models were: age and sex (both forced into the model), body mass index (BMI), cigarette smoking, adult onset diabetes mellitus, total cholesterol, high-density lipoprotein (HDL) cholesterol, lipid-lowering treatment, hypertension treatment, systolic blood pressure, diastolic blood pressure, prevalent CVD, and fasting glucose. Covariates with $p<0.05$ in the multivariable model are reported. We used generalized estimating equations (GEE) to account for the familial correlation within our dataset.

Association of biomarker level with coronary artery calcification and incident CVD and mortality—We performed Cox proportional hazard models with log-OPG and log-RANKL (each biomarker separately) as predictor variables and with death (all-cause mortality) and incident CVD (defined as above) as two separate outcomes. We conducted age- and sex-adjusted and multivariable models adjusting for age, sex, smoking, diabetes mellitus, total/HDL cholesterol, hypertension treatment, systolic and diastolic blood pressure, lipid-lowering treatment, fasting serum glucose, and C-reactive protein (CRP) levels. Models for mortality were also adjusted for prevalent CVD at baseline. Furthermore, we analysed a multivariable-adjusted model with both OPG and RANKL in the same model. We additionally ran a separate analysis for the combined endpoint of MI, vascular death and stroke. Given the skewed distribution of both biomarkers we also analysed the age- and sex-adjusted as well as multivariable-adjusted hazard ratios for CVD and mortality stratified by quartiles of OPG and RANKL, using the first biomarker quartile as the referent. Logistic regression models were used to assess the cross-sectional associations of log-OPG and log-RANKL with coronary artery calcification. We examined age- and sex-adjusted models and multivariable-adjusted models (same covariates as in the multivariable-adjusted model described above).

Results

Baseline characteristics of our sample are displayed in Table 1. Age- and sex-adjusted correlation between log-OPG and log-RANKL was small to modest ($r = -0.10$; $p=<0.0001$), which is in good agreement with previous studies.[14, 20] The distributions of OPG and RANKL are shown in online Figure 1 and 2.

Clinical correlates of OPG and RANKL

OPG was higher in women as compared to men and positively related to age, smoking, diabetes, systolic blood pressure, serum glucose and prevalent CVD. Inverse associations were observed with total cholesterol, diastolic blood pressure and the intake of lipid-lowering medication (Table 2A). Replacing systolic and diastolic blood pressure by pulse pressure revealed a positive association with OPG ($\beta=0.0021$; SE 0.0003; $p <0.0001$).

RANKL levels were likewise higher in women as compared to men and displayed inverse associations with age, smoking status, diabetes, HDL cholesterol, and antihypertensive treatment (Table 2B).

Association of circulating OPG and RANKL levels with incident CVD and mortality

During a mean follow-up of 4.6 years, 143 participants (of 3084 without prevalent CVD at baseline) developed incident CVD and 235 participants died. In age- and sex-adjusted models as well as in multivariable-adjusted models, accounting for the familial correlation within our dataset, OPG concentrations were significantly positively related to incident CVD and mortality (Table 3, $p=0.017$ for CVD, $p=0.005$ for mortality), whereas RANKL levels were not (Table 3; $p>0.05$ for CVD and mortality). Additional adjustment for BMI did not alter the results.

OPG-quartiles were associated with increased hazards for CVD (Q4 vs. Q1, HR: 1.69; 95% CI, 0.98 to 2.92; $p=0.094$ for trend across OPG-quartiles) and mortality (Q4 vs. Q1, HR: 1.77, 95% CI, 1.04 to 3.00; $p=0.033$ for trend across OPG-quartiles), even after multivariable adjustment. The association of OPG-quartiles with CVD failed to reach statistical significance. In secondary analyses, OPG levels, but not RANKL levels, were associated with the combined endpoint stroke, MI, and vascular death (online Table 1).

Association of circulating OPG and RANKL with coronary artery calcification

Compared to individuals in the first OPG-quartile, participants in the top quartile had increased odds for prevalent coronary artery calcification (OR: 1.61; 95% CI, 1.09 to 2.40; p=0.018 in age-and sex adjusted models). However, multivariable adjustment rendered this association non-significant. Likewise, in multivariable-adjusted models, the prevalence of coronary artery calcification increased with OPG-quartiles, although the difference did not reach statistical significance (p=0.20 for trend across quartiles; Table 4). No association of RANKL-quartiles with coronary artery calcification was observed (Table 4).

Discussion

Principal findings

Our main findings were: (1) circulating OPG concentrations predicted incident CVD and mortality independent of established risk factors, whereas circulating RANKL levels did not; (2) OPG levels displayed positive whereas RANKL concentrations showed inverse cross-sectional associations with multiple CVD risk factors; (3) in a moderate-sized subset with CT-measured coronary artery calcification, the prevalence of coronary artery calcification showed a weak non-significant increase with OPG quartiles and was not related to RANKL quartiles.

In the context of the current literature

Clinical correlates of OPG and RANKL—The inverse correlation of OPG and RANKL has been reported in previous studies.14, 20 We observed positive associations of OPG levels with multiple CVD risk factors including age, smoking, diabetes, systolic blood and pulse pressure, prevalent CVD, and fasting glucose levels. In addition, we observed inverse associations with total cholesterol, lipid-lowering treatment and diastolic blood pressure as well as higher OPG levels in women as compared to men. These findings are in good agreement with results from other community-based cohorts including the Dallas Heart Study21 and the Bruneck Study.[13] Although the findings for some individual risk factors were not entirely consistent across the three community-based cohorts (Dallas, Bruneck, Framingham), the overall picture indicates that OPG is cross-sectionally related to multiple CVD risk factors. This indicates that OPG either serves as a marker for increased CVD risk factor burden or might be along the causal pathway in the development of atherosclerotic lesions (see below).

Data regarding the clinical correlates of RANKL are sparse. In the community-based Bruneck study (n=906), RANKL levels were not related to age, sex, hormone replacement therapy, smoking or diabtes.22 In the EPIC Norfolk study, lipid and blood pressure levels, and age were slightly different by RANKL quartiles (reaching statistical significance) in men, but no consistent directionality across quartiles could be observed. CRP levels differed by RANKL quartiles in women.16 Certain patient samples suggested no association of RANKL with traditional CV risk factors; e. g. in a small series of male patients referred for coronary angiography (n= 346).20 However, the moderate to small size of these samples might have limited the power to detect moderate effects in the latter studies. In our larger sample from the general population, including more than 3200 participants, we observed higher RANKL levels in women and modest inverse associations of RANKL with age, smoking, diabetes, antihypertensive treatment (a marker of chronic hypertension) and HDL cholesterol, indicating that RANKL might reflect a more favorable CVD risk profile.

OPG and RANKL levels and incident CVD and mortality—Our observations of strong, positive associations of baseline OPG levels with incident CVD and mortality independent of traditional CVD risk factors are in excellent agreement with previous smaller

sized epidemiological and clinical studies. In 2001, the first study reporting a positive association of OPG with all-cause and cardiovascular mortality and incident diabetes was published.15 Although no association with stroke was noted in that moderate sized cohort (n=490),[15] Kiechl and colleagues observed a significant association of OPG with 10-year incidence of CVD (defined as myocardial infarction, stroke, transient ischemic attack, peripheral artery disease) and vascular mortality (due to myocardial infarction, stroke, ruptured aneurysm, sudden cardiac death) as well as with the initiation and progression of carotid atherosclerosis.13 In addition, higher OPG levels were observed in patients with unstable angina,6 with acute myocardial infarction,10 and with significant coronary artery stenosis.11 Furthermore, baseline OPG levels predicted long-term mortality and heart failure hospitalisations in patients with acute coronary syndromes.[12] Recently, Semb and colleagues demonstrated that baseline levels of OPG (but not baseline RANKL) were associated with incident coronary events in the EPIC Norfolk study.[16]

It is, however, not clear whether OPG is causally related to atherosclerotic disease progression and acute coronary events or rather serve as a marker of atherosclerotic disease burden with high OPG levels being a modulatory response to subclinical CVD.[13, 15] In vitro data suggest involvement of OPG in apoptosis and inflammation.[5] OPG is expressed in different cell types within the vessel wall, including endothelial cells and vascular smooth muscle cells, and its expression is modulated by pro-inflammatory cytokines.[5] Furthermore, OPG was detected in atherosclerotic lesions and enhances survival of endothelial cells.[7] On the other hand, animal models and experimental data indicated that OPG has a rather protective effect against vascular calcification[8] and that higher OPG levels in patients with CVD might be rather a compensatory response to atherosclerotic disease,[15] favoring the interpretation of OPG being a marker of CVD rather than a risk factor.

Recently, therapeutic drugs targeting the OPG/RANKL pathway have been developed and tested in randomized clinical trials.[23, 24] CVD side effects have not been different between the treatment and the placebo group in these trials.[23, 24]

In the Bruneck study, baseline levels of RANKL were found to predict incident CVD,[14] whereas RANKL levels were not associated with all-cause mortality or CVD in our analyses. One potential explanation for this discrepancy is that the study by Kiechl and colleagues focused on acute vascular syndromes, while the present analyses used a mixed endpoint including both acute vascular syndromes and conditions like stable CHD and heart failure. Another explanation is the larger coefficient of variation for RANKL in our dataset, limiting our ability to detect modest associations for RANKL. To further elucidate their associations with subclinical disease, we also explored the association of circulating OPG and RANKL with coronary artery calcification, measured by MDCT.

OPG and RANKL and coronary artery calcification—We observed a weak association of OPG with coronary artery calcification in a moderate-sized (n=1264) subsample with higher odds in the 4th OPG quartile compared to the 1st quartile. This is in good agreement with finding from previous epidemiological and clinical studies reporting that OPG was positively related to coronary artery calcification in the general population[21] as well as in selected patients group (e. g. patients with diabetic nephropathy).[25] Likewise, OPG was positively related to the extent of aortic calcification in patients with peripheral artery disease26 or patients on hemodialysis.27 Given its positive association with several cardiovascular risk factors, the association of OPG with coronary artery calcification might be one potential mechanism leading to CVD events and explaining the association of OPG with clinically overt CVD and mortality in our and previous samples.[10, 13, 15]

Strength and limitations

The large (n>3200) sample, the community-based prospective design, the availability of coronary calcification in a moderate-sized subsample of 1264 individuals, and the rigorous assessment of clinical covariates and endpoints strengthen our investigation. Some limitations, however, merit consideration. Circulating OPG and RANKL concentrations might not adequately reflect concentrations on the tissue level. Because our data were obtained in a middle-aged cohort of European descent, the generalizability of our finding to other age groups or ethnicities is unclear. Furthermore, biomarker levels were only measured once in each participant, which might have biased us towards the null hypothesis of no association between biomarkers and the outcome variables. We only had a modest number of events and hence our power to detect associations was limited. Also, we combined CVD events because we lacked power to examine specific events; we acknowledge that the pathophysiology of the different CVD outcomes may differ. As noted above, an observational study design cannot definitively examine whether biomarkers are causally related to clinical or subclinical CVD. We cannot exclude the possibility of residual confounding. The average time interval between biomarker measurement and the assessment of coronary artery calcification was 4.2 years, so the design was not purely cross-sectional, nor prospective. However, since biomarkers were measured before coronary artery calcification was determined and since calcifications usually evolve progressively over the adult life course, this time lag should not necessarily weaken our ability to detect associations.

In conclusion, we observed a positive association of circulating OPG (but not RANKL) with incident CVD and mortality in the thus far largest community-based sample. In addition, OPG was associated with a rather adverse CVD risk factor profile whereas RANKL was related to a more favorable risk factor profile. Whether OPG serves as a marker of adverse CVD risk factor burden and subclinical disease or is along a causal pathway of vascular remodeling and CVD merits further investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics of the study sample (n=3250).

Clinical features	
Women, %	54
Age, yrs.	61 (9)
Systolic BP, mm Hg	127 (19)
Diastolic BP, mm Hg	74 (10)
Antihypertensive treatment, %	33%
Body mass index, kg/m^2	28.2 (5.3)
Current cigarette smoking, %	13%
Diabetes, %	13%
Glucose, mg/dL	104 (27)
Lipid-lowering treatment, %	20%
Prevalent CVD, %	5.1% (n=166)
Biochemical features	
Total/HDL cholesterol	4.1 (1.3)
Total cholesterol, mg/dL	200 (37)
HDL cholesterol, mg/dL	54 (17)
Creatinine, mg/dL	0.86 (0.20)
CRP, mg/dL	2.17 (1.02,5.13)
Log-CRP	0.77 (0.02,1.64)
OPG, pmol/L	5.40 (4.45,6.49)
Log-OPG	1.69 (1.49,1.87)
RANKL, pmol/L	0.05 (0.02,0.14)
Log-RANKL	−3.00 (−3.91,−1.96)

BP, blood pressure; CVD, cardiovascular disease; CRP, C-reactive protein; HDL, high-density lipoprotein, OPG,osteoprotegerin; RANKL, receptor activator of nuclear factor–kappa-B ligand.

Data are mean (standard deviation), except for CRP, Log-CRP, RANKL, OPG, Log-RANKL and Log-OPG, where median (quartile 1, quartile 3) are provided.

Table 2

A. Clinical correlates of OPG (osteoprotegerin), adjusting for the familial structure within our dataset (n=3249).

	Beta Estimate	Standard Error	P value
Age	0.011	0.0007	<0.0001
Female sex	0.082	0.010	<0.0001
Smoking	0.083	0.013	<0.0001
Diabetes	0.064	0.018	0.0005
Total cholesterol	−0.0006	0.0001	<0.0001
Lipid-lowering treatment	−0.052	0.013	<0.0001
Systolic BP	0.002	0.0004	<.0001
Diastolic BP	−0.003	0.0007	<0.0001
Prevalent CVD	0.066	0.016	<0.0001
Glucose	0.0007	0.0002	0.0006

B. Clinical correlates of RANKL, adjusting for the familial structure within our dataset (n=3196).

	Beat Estimate	Standard Error	P value
Age	−0.008	0.002	0.0004
Female sex	0.104	0.044	0.019
Smoking	−0.195	0.054	0.0003
Diabetes	−0.193	0.056	0.0006
HDL cholesterol	−0.004	0.001	0.0024
Antihypertensive treatment	−0.123	0.042	0.0037

BP, blood pressure; CVD, cardiovascular disease.
The regression coefficients indicate the increase in log-OPG per one-unit increment in the predictor variable; for binary traits that corresponds to the presence vs. absence of the trait.
The following variables were not significant (p>0.05) in the forward selection model:
Body mass index, high-density lipoprotein cholesterol, and hypertension treatment
The set of significant variables explained 24% of the inter-individual variation in log-OPG levels.

RANKL, receptor activator of nuclear factor–kappa-B ligand; HDL, high-density lipoprotein
The regression coefficients indicate the increase in log-RANKL per one-unit increment in the predictor variable; for binary traits that corresponds to the presence vs. absence of the trait.
The following variables were not significant (p>0.05) in the forward selection model:
Body mass index, total cholesterol, lipid-lowering treatment, systolic blood pressure, diastolic blood pressure, prevalent cardiovascular disease, and fasting glucose. The set of significant variables explained 2% of the inter-individual variation in log-RANKL levels.

Table 3

Association of OPG and RANKL with all-cause mortality and incident CVD after adjustment for traditional risk factors, correlates of OPG and RANKL, and for the familial correlation within our dataset.

Model	Hazards Ratio per 1-SD increment in log-RANKL (95% CI)	P	Hazards Ratio per 1-SD increment in log-OPG (95% CI)	P
Incidence of CVD (events/at risk)	143/3041		143/3084	
Age- and sex-adjusted model	1.03 (0.89, 1.21)	0.67	1.28 (1.08, 1.51)	0.0048
Multivariable-adjusted model*	1.02 (0.86, 1.21)	0.82	1.27 (1.04, 1.54)	0.017
Multivariable-adjusted with OPG and RANKL	1.04 (0.88, 1.23)	0.68	1.28 (1.05, 1.55)	0.014
All-cause mortality (deaths/at risk)	232/3202		235/3249	
Age-, sex- and prevalent CVD-adjusted model	0.89 (0.78, 1.01)	0.07	1.31(1.14, 1.50)	0.0002
Multivariable-adjusted model*	0.92 (0.80, 1.05)	0.22	1.25 (1.07, 1.47)	0.005
Multivariable-adjusted with OPG and RANKL	0.92 (0.80, 1,06)	0.23	1.25 (1.07, 1.46)	0.006

RANKL, receptor activator of nuclear factor–kappa-B ligand; OPG, osteoprotegerin; CI, confidence interval; SD, standard deviation, CRP, Creactive protein,

*adjusted for age, sex, smoking, diabetes mellitus, total/HDL cholesterol, hypertension treatment, systolic blood pressure, CRP, diastolic blood pressure, lipid-lowering medication and serum glucose. Models for all-cause mortality were additionally adjusted for prevalent CVD.

Table 4

Prevalence of and odds ratios (OR) for coronary artery calcification (CAC) stratified by quartiles of OPG and RANKL

OPG-Quartile	Prevalence of CAC	OR (95% CI) for CAC	p for trend
1	24%	Referent	
2	27%	1.27 (0.87,1.84)	0.20
3	27%	1.30 (0.89,1.91)	
4	28%	1.32 (0.87,2.00)	
RANKL-Quartile	**Prevalence of CAC**	**OR (95% CI) for CAC**	**p for trend**
1	26%	Referent	
2	29%	1.23 (0.85,1.77)	
3	28%	1.29 (0.89,1.85)	0.81
4	23%	0.93 (0.63,1.35)	

RANKL, receptor activator of nuclear factor–kappa-B ligand; OPG, osteoprotegerin; CI. Confidence interval; OR and p are adjusted for for age, sex, smoking, diabetes mellitus, total/HDL cholesterol, hypertension treatment, systolic blood pressure, CRP, diastolic blood pressure, lipid-lowering medication and serum glucose