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Serum osteoprotegerin levels and long-term prognosis in patients with stable angina pectoris

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ABSTRACT

Objectives: Osteoprotegerin (OPG) is a member of the tumor necrosis factor superfamily with pleiotropic effects on bone metabolism, endocrine function and the immune system. Circulating OPG levels are elevated in cardiovascular disease (CVD). We assessed serum OPG as predictor of long-term prognosis in patients with suspected stable angina pectoris (SAP) undergoing elective coronary angiography.
Methods: Samples were obtained from 1025 patients (median [25th, 75th percentile] age 62 [54, 70] years, 71.9% men). At inclusion, 43.2% of patients had single or double vessel disease, whereas 34.3% had triple vessel disease.
Results: During a median follow-up of 73 months, 11.0% of patients died, 5.9% died from CVD and 10.0% experienced an acute myocardial infarction (MI). In univariable analyses, strong associations were observed between OPG concentrations and all-cause mortality, CVD mortality and the incidence of MI (fatal or nonfatal). However, adjustment for conventional risk factors attenuated the risk estimates which were no longer significant, except for the subgroup with levels above the 90th percentile. For decile 10 versus deciles 1–9 of serum OPG, the following multivariable hazard ratios (95% confidence intervals) were observed: All-cause mortality: 1.94 (1.18, 3.18), $p = 0.01$; CVD mortality: 2.29 (1.16, 4.49), $p = 0.02$; and MI: 1.76 (1.02, 3.06), $p = 0.04$.
Conclusion: In patients with SAP, elevated serum OPG is associated with increased risk of all-cause mortality, CVD mortality and MI, but independent effects are mainly confined to levels above the 90th percentile.

1. Introduction

Several studies have demonstrated a relationship between bone pathology and vascular disease [1,2]. The frequent coexistence of osteoporosis and features of atherosclerosis has lead to the hypothesis of common pathways, which negatively affect both bone metabolism and vasculature. During the recent years, atherosclerosis has been known as a systemic inflammatory process involving immune and vascular cells. This inflammatory process could represent a link between disturbed bone homeostasis, vascular calcification and atherogenesis.

Osteoprotegerin (OPG) is a secretory protein that belongs to the tumor necrosis factor (TNF) receptor superfamily. OPG inhibits osteoclastogenesis by binding the receptor activator of nuclear factor κB ligand (RANKL), acting as a decoy receptor to competitively inhibit RANKL interaction with its receptor RANK [3]. Recently, the OPG/RANKL/RANK axis has been implicated in various inflammatory and matrix degrading responses [4,5], and has also been linked to cardiovascular disease (CVD) [5–7].

In observational studies, elevated circulating OPG levels have been associated with increased prevalence and severity of coronary artery disease (CAD) [8], peripheral vascular disease [9], cerebrovascular disease [10], and vascular dementia [11]. Circulating OPG levels are increased in patients with acute coronary syndrome (ACS) [5], and enhanced expression has been found within symptomatic carotid plaques [12]. Elevated serum OPG levels were associated with increased risk of CVD in apparently healthy individ-

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uals [13]. In a relatively small study sample, OPG predicted survival in patients with heart failure (HF) after acute myocardial infarction (MI) [14], and has also been shown to predict long-term mortality in patients with ACS [15] and in patients with ischemic stroke [16].

OPG has not previously been evaluated as a prognostic marker in patients with stable angina pectoris (SAP), and there is in general a paucity of biomarkers applicable for clinical risk stratification of this patient group [17]. We therefore assessed the associations of serum OPG level to long-term risk of all-cause mortality, CVD mortality and MI in a large cohort of SAP patients.

2. Methods

2.1. Patient population

The study population consisted of 1025 consecutively recruited patients with suspected SAP, who underwent elective coronary angiography, in the period of January 2000 to June 2001 at the Department of Heart Disease, Haukeland University Hospital in Bergen, Norway. Participants represented about 99% of all patients that were referred to coronary angiographic examination for stable chest pain in this period.

Patients completed a self-administered questionnaire that provided information about medical history, risk factors and medications. History of hypertension and heart failure indicate subjects being treated for these co-morbidities according to clinical criteria. Diabetes mellitus includes both type 1 and 2. Smokers include currently smokers and those reporting having quit within the last 4 weeks, since relapse rates usually are very high the first month after smoking cessation [18]. Left ventricular ejection fraction (LVEF) was determined by ventriculography or echocardiography.

For all patients, information from the questionnaires was checked against medical records. The study was approved by the regional Committee for Medical and Health Research Ethics and the Norwegian Data Inspectorate. Written, informed consent was obtained from all the participants.

2.2. Angiographic evidence of CAD

Coronary angiograms were performed by cardiologists. Prevalence of CAD was defined as a diameter stenosis of $\geq$50% in any of the main coronary arteries (left ascending artery [LAD], circumflex artery [CX] and right coronary artery [RCA]). The extent of significant CAD was scored as no CAD, single vessel disease, double vessel disease or triple vessel disease, according to the number of main vessels with stenosis. Presence of left main-stem artery stenosis with no RCA stenosis was classified as double vessel disease or as triple vessel disease if RCA was hypoplastic.

2.3. Follow-up and clinical endpoints

Patients were followed from the time of baseline angiography throughout the year 2006. Information was collected from the Cause of Death Registry at Statistics Norway and from the Western Norway Cardiovascular Registry, which contains all CVD discharge diagnoses from the patient-administrative systems at the hospitals in Western Norway. Data from these registries were checked against hospital medical records. Study endpoints were all-cause mortality, CVD mortality and MI. A person was considered diseased from CVD if the underlying cause of death was coded as International Statistical Classification of Diseases, 10th Revision (ICD-10), codes I00 to I99 (which also included fatal MIs), or code R96. The MI endpoint encompassed nonfatal and fatal events classified according to the diagnostic criteria of the revised definition published in 2000 [19]. This endpoint also included ICD-10 codes I46.1 and R96 ("Sudden cardiac death" and "Sudden death"). We excluded MIs occurring within 24 h after coronary angiography, percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG), as these were considered as procedure-related. All events were adjudicated by members of the endpoints committee, who were unaware of the patients' serum OPG levels.

2.4. Biochemical analyses

Blood samples were collected before coronary angiography and serum aliquots immediately frozen at −80 °C until analysed in 2005. All samples were treated in a similar manner. Serum OPG was quantified by an enzyme immunoassay using commercially available matched antibodies (R&D Systems, Minneapolis, MN). The intra- and inter-assay coefficients of variation (CV) were 3.6% and 10.6%, respectively. The sensitivity, defined as the mean ± 3 SD of the 0 standard, was calculated to be 15 pg/mL [20]. Serum C-reactive protein (CRP) was determined by an ultra sensitive immunoassay, with a lower detection limit of 0.17 mg/L, applying the Behring nephelometer II system (CV 8.1–11.4%; N Latex CRP mono, Behring Diagnostics, Marburg, Germany). Routine laboratory analyses were performed immediately by standard assays at the Department of Clinical Biochemistry, Haukeland University Hospital, Bergen, Norway. Samples were analysed in duplicate by laboratory personnel blinded to the clinical outcome of patients.

2.5. Statistical analyses

Continuous variables are reported as means (SD) or medians (25th, 75th percentile), and categorical variables as percentages. Differences across quartiles of OPG level were explored using linear regression for continuous variables and logistic regression for categorical variables. Values of creatinine, CRP and OPG showed a right skewed distribution and were therefore logarithmically transformed before analyses, and back transformed to the original scale when presented. Associations between continuous variables were assessed by Spearman rank correlation.

Cox regression was applied to calculate hazard ratios for each quartile increment of serum OPG and for OPG as a dichotomous variable with cut-off set at the 90th percentile. These stratifications were selected after inspection of generalized additive model plots [21] which revealed nearly linear increments in risk of events along with increasing OPG levels in crude analyses, whereas after multivariable adjustment, the increased risk was predominantly seen at OPG levels above the 90th percentile. The multivariable model included age, gender, hypertension, smoking status, diabetes mellitus, creatinine, high density lipoprotein (HDL) cholesterol, CRP, angiographic extent of CAD, LVEF, treatment after baseline coronary angiography (none, medications only, PCI, CABG), and use of loop diuretics. Covariates for multivariable adjustment were selected after calculating relations between baseline risk factors, OPG concentrations (quartiles) and mortality in unadjusted analyses. Variables were subsequently included if $p < 0.2$. We performed Schoenfeld's test to ensure that assumptions of proportional hazards were not violated. Unadjusted cumulative survival for decile 10 versus deciles 1–9 of serum OPG were visualized by Kaplan–Meier curves.

We tested the incremental prognostic value of OPG in relation to study endpoints by calculation of areas under receiver operating characteristics (ROC) curves and by the determination of net reclassification improvement (NRI) [22]; using a follow-up time of 64 months. For the NRI analyses, participants were classified into three risk categories (<10%, 10% to <20%, or 20% or greater risk of events during follow-up).

Statistical power was assessed on the basis of a 2-sided χ^2 test comparing deciles 1–9 (combined) versus decile 10 (Sample Power