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# Eosinophil cationic protein: A new biomarker of coronary atherosclerosis

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ABSTRACT

*Aims:* Coronary atherosclerosis is a chronic inflammatory disease, but different inflammatory biomarkers may reflect different phases of atherosclerotic plaque evolution. We aimed at assessing the role of eosinophil cationic protein (ECP), a sensitive marker of eosinophil activation, and C-reactive protein (CRP) in coronary artery disease (CAD).
*Methods and results:* Consecutive anginal patients with angiographic evidence of CAD [stable angina (SA) or non-ST-elevation acute coronary syndrome (NSTE-ACS)], or with angiographically normal coronary arteries (NCA) were enrolled. The severity of CAD was graded according to Bogaty's score and coronary lesion morphology was defined as smooth or complex. Baseline ECP and high sensitivity CRP were measured in all patients. Of 198 patients (64 ± 10 years, male 74%), 91 had SA, 57 had NSTE-ACS and 50 had NCA. ECP levels were significantly higher in SA [30 μg/L (13.8–46.9), $p < 0.001$] and NSTE-ACS [21.8 μg/L (5.5–46.3), $p = 0.016$] compared to NCA [9.7 μg/L (6.1–13.6)], without significant difference between SA and NSTE-ACS ($p = 0.45$). CRP levels were significantly higher in NSTE-ACS [2.38 mg/L (1.11–11.94)] compared to SA [1.48 mg/L (0.82–2.83), $p = 0.03$], and NCA [1.09 mg/L (0.8–2.1), $p < 0.001$], without significant difference between SA and NCA ($p = 0.20$). The addition of ECP to main cardiovascular risk factors improved the area under the curve from 0.88 to 0.92, $p = 0.007$ for the angiographic diagnosis of CAD; further addition of CRP increased the area to 0.94, $p = 0.014$. At multiple linear regression analysis ECP levels independently predicted CAD severity ($p = 0.001$), whereas CRP levels independently predicted lesion complexity ($p = 0.01$).
*Conclusions:* Our study shows that ECP is a marker of CAD and that different inflammatory biomarkers reflect different phases of atherosclerotic plaque evolution.


## 1. Introduction

Leukocyte recruitment and expression of proinflammatory cytokines characterize all steps of atherothrombosis [1]. Recent observations suggest that eosinophils may play a role in coronary atherosclerosis. Indeed, prospective studies have consistently shown an association between eosinophil count and increased risk for future cardiovascular events [2,3]. Furthermore, eotaxin, an eosinophil-specific chemoattractant, is overexpressed in human atherosclerotic lesions [4] and patients with coronary artery disease (CAD) show higher circulating levels of eotaxin as compared to healthy controls [5,6]. Accordingly, a non-conservative polymorphism in the eotaxin gene [7] together with sequence variants affecting eosinophil count [8] have recently been associated with an increased risk of myocardial infarction.

Eosinophil cationic protein (ECP) is a zinc-containing, highly cationic protein, stored in the peroxidase-positive and negative eosinophil granules which is secreted through priming by various triggers, such as immunoglobulins and complement components [9]. Several studies have shown that the measurement of ECP in most biological fluids may be used as a marker of eosinophil activity and turnover, and that increased ECP serum levels are related to the presence, activity and severity of asthma, atopic disorders, and other immune diseases, such as rheumatoid arthritis, psoriasis, and adult celiac disease [10]. The role of eosinophil activation, as assessed by ECP serum levels, in coronary atherosclerosis severity has not been investigated yet. Among non-specific markers of inflammation, C-reactive protein (CRP) is the most investigated and has widely been associated with an increased risk of future cardiovascular events both in primary and secondary prevention studies [11–14]. However, the association of CRP levels with coronary

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atherosclerotic burden is controversial [15–18], whereas its association with coronary instability has consistently been reported in several studies [19–21].

In this study, we assessed the association of ECP and CRP with coronary atherosclerotic burden on the one hand and with coronary instability on the other.

## 2. Methods

### 2.1. Patient population

Consecutive patients undergoing coronary angiography because of chest pain between May and June 2008 were prospectively included in the study. Patients found to have angiographically normal coronary arteries were classified as patients with normal coronary arteries (NCA), whereas patients showing detectable atheroma at angiography were classified as patients with coronary artery disease (CAD). Non-ST-elevation acute coronary syndrome (NSTE-ACS) was defined as chest pain at rest in the last 48 h preceding the admission associated with evidence of transient ST segment depression on 12-lead ECG and normal (unstable angina) or elevated (non-ST-elevation myocardial infarction) serum troponin T levels. Stable angina (SA) was defined as angina on effort with a stable pattern of symptoms for at least the last 6 months prior to admission. Overall 270 patients were initially screened for the study. Exclusion criteria were ST-elevation myocardial infarction ($n = 26$ patients), in-stent restenosis ($n = 15$), culprit lesion in a saphenous vein graft ($n = 8$), severe chronic heart failure (NYHA class III-IV) ($n = 8$ patients), severe heart valve disease ($n = 3$ patients), acute and chronic infections ($n = 2$ patients), autoimmune diseases ($n = 1$ patient), liver diseases ($n = 1$ patient), neoplasia ($n = 2$ patients), evidence of immunologic disorders ($n = 1$ patient), use of anti-inflammatory or immunosuppressive drugs ($n = 3$ patients), recent (<3 months) surgical procedures or trauma ($n = 2$ patients). Patients with a history of allergy were not excluded from the study ($n = 8$ patients). In all patients cardiovascular risk factors were carefully examined, including history of CAD (any previous diagnosis of stable or unstable coronary syndrome), family history of early CAD (first degree relative with a history of myocardial infarction <60 years), diabetes (fasting blood glucose >126 mg/dL or treated diabetes), hypercholesterolemia (total cholesterol >200 mg/dL or treated hypercholesterolemia), smoking, and hypertension (systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or treated hypertension).

Body Mass Index (BMI) and laboratory data including serum glucose and lipid profile were also collected. Medications taken on admission were recorded. After a complete explanation of the aims and details of the study, all patients gave their informed consent before entering the study.

### 2.2. Coronary angiographic analysis

Two expert angiographers (G.N. and F.B.), who were blinded to the laboratory values, evaluated all angiographic images to assess presence, severity and extent of CAD and the morphology of all coronary artery stenoses causing ≥30% reduction in lumen diameter. Any disagreement between the two angiographers was resolved by consensus; when consensus could not be reached, a third experienced angiographer (M.C.) assessed and classified the coronary lesions. NCA was defined as the lack of any detectable atheroma or parietal irregularities at angiography. To evaluate coronary atherosclerosis by angiography, the score developed by Bogaty et al. was applied [22]. Briefly, the Bogaty's score assesses disease severity and extent. Severity of disease (stenosis score) refers to the total number of ≥50% narrowings in all vessels of the angiogram. A maximum of three stenoses was permitted per coronary arterial segment. Extent of disease (extent index) is obtained by dividing the extent score of the entire coronary arterial tree by the number of analyzed segments. A segment is scored 0 if it appeared angiographically normal, 1 if ≤10% of its length appeared abnormal, 2 if >10% up to 50% of its length was abnormal, and 3 if >50% of its length was abnormal. Since there were 15 segments considered, the extent index could vary from 0 to 3. Stenosis morphology was assessed as previously reported [23,24]. Briefly, stenoses were considered to be complex or smooth. Complex lesions were diagnosed according to the presence of at least one of the following features: (1) irregular morphology or scalloped borders, or both; (2) overhanging or abrupt edges perpendicular to the vessel wall; (3) ulceration (i.e., outpouchings within the stenosis); (4) the presence of filling defects consistent with intracoronary thrombus [25]. Coronary stenoses without any of the complex features were classified as smooth lesions. The percentage of complex lesions per patient was calculated.

### 2.3. Blood samples and laboratory assay

Venous blood samples were drawn just prior to coronary angiography and then centrifugated in appropriate tubes; aliquots of serum were stored at −80 °C until assayed. ECP was measured by an highly specific ELISA (UniCap, Phadia, Uppsala, Sweden) and expressed as μg/L. For ECP serum levels, bounds of detection were 0.5–200 μg/L and interassay coefficient of variation was 4% [26,27]. CRP was measured by an ultrasensitive nephelometric method (DADE-Behring Latex BN-2), with a lower detection limit of 0.2 mg/L. Biological measurements were available for all enrolled patients.

### 2.4. Statistical analysis

The distribution of continuous variables was assessed by visual inspection of frequency histograms and with the use of the Shapiro–Wilk test. Continuous variables were expressed as mean ± standard deviation (SD) or median (interquartile range), if they followed a normal or non-normal distribution, respectively. Continuous variables among the three groups of subjects in the study population (NSTE-ACS, SA, NCA) were compared with ANOVA or Kruskal–Wallis test; unpaired *t*-test or Mann–Whitney *U*-test were used for the comparison between two groups; categorical variables were compared using the chi square test or Fisher's exact test, as appropriate. Bonferroni's correction for multiple comparison was applied and a Bonferroni-adjusted-*p*-value was reported. ECP and CRP levels were adjusted for age, sex and the main cardiovascular risk factors (i.e., diabetes, family history of CAD, hypercholesterolemia, hypertension and smoking) using ANCOVA analysis. For this purpose, ECP and CRP levels underwent logarithmic transformation to meet the ANCOVA assumptions; bootstrap resampling with 5000 replications of the *p*-value was performed, owing to the presence of small residual skewness of the residuals distribution, calculating the bias corrected and accelerated (BCa) estimate of the 95% confidence interval (CI) of the *p*-value; the Bonferroni's correction was applied setting the BCa CI at $100 \times (1 - 0.05/3)$ level; weighted least squares were the estimator to correct for heteroskedasticity in ANCOVA analysis of CRP levels.

We then assessed the classification performance of several models for the diagnosis of CAD: model 1 including age, sex and the main cardiovascular risk factors (diabetes, family history of CAD, hypercholesterolemia, hypertension and smoking); model 2 including ECP only as continuous covariate; model 3 including ECP as continuous covariate and the covariates in model 1; model 4 including ECP, CRP as continuous covariates and the covariates in model 1.