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Expression Characteristics of ECP in Coronary Artery Calcification and Its Correlation with IL-4 and IL-13

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Abstract

Background

Coronary artery disease (CAD) is a major cause of global mortality, and coronary artery calcification (CAC) is a key marker of atherosclerotic burden. Eosinophil cationic protein (ECP), a granule protein released by activated eosinophils, has been implicated in inflammatory processes. However, its role in CAC and cardiovascular events (CVE) remains unclear.

Objectives

This study aimed to compare the expression of eosinophil cationic protein (ECP) between patients with coronary artery disease (CAD) and healthy controls, and to explore the correlations of ECP levels with coronary artery calcification (CAC) and cardiovascular events (CVE).

Methods

A total of 151 subjects were enrolled, including 86 CAC patients and 65 healthy controls. Enzyme-linked immunosorbent assay (ELISA) was used to detect the levels of ECP, interleukin-4 (IL-4), and interleukin-13 (IL-13) in whole blood. The severity of CAC was evaluated by Agatston score based on coronary computed tomography (CT). All subjects were followed up regularly to record the incidence of CVE, and statistical analyses were performed using univariate/multivariate linear regression, Cox proportional hazards model, and Kaplan-Meier survival curve with log-rank test.

Results

showed that ECP levels were significantly higher in CAD patients than in healthy controls ($P < 0.05$), and notably elevated in the CAC group compared with the non-CAC group ($P < 0.05$). ECP expression increased with the aggravation of CAC, with the highest level observed in patients with severe CAC ($P < 0.05$). IL-4 and IL-13 levels showed no significant differences between groups ($P > 0.05$). Multivariate linear regression analysis identified ECP, age, brain natriuretic peptide (BNP), and blood urea nitrogen (BUN) as independent influencing factors of CAC score (all $P < 0.05$). Univariate Cox regression indicated that CAC score, ECP, BNP, and hypertension were associated with an increased risk of CVE (all $P < 0.05$), while only hypertension remained a significant risk factor in multivariate analysis. Kaplan-Meier curves revealed that patients with high ECP levels (> 11.09 pg/ml) or severe CAC had a significantly higher risk of CVE (both $P < 0.001$).

Conclusions

ECP is closely correlated with CAC and may directly participate in the vascular calcification process through BMPR-mediated signaling pathways. As a specific marker of eosinophil activation, ECP holds promise as a novel biomarker for risk assessment and a potential therapeutic target in CAC.

Introduction

Coronary artery calcification (CAC) is a manifestation of coronary atherosclerosis .With the intensification of population aging and changes in dietary structure, CAC has become increasingly prevalent among middle-aged and elderly populations[1, 2]. Studies have shown that the detection rate of CAC can be as high as 70%-85% in individuals over 60 years of age. In clinical practice, coronary artery calcification (CAC) has been shown to be closely associated with both the occurrence and progression of adverse cardiovascular events, including myocardial infarction, arrhythmias, heart failure, and sudden cardiac death.Accumulating evidence indicates that vascular calcification (VC) in patients with coronary artery disease (CAD) is not merely a passive consequence of lipid, calcium, fibrous tissue, and cellular debris accumulation within the arterial intima, but rather an actively regulated process driven by osteogenic signaling pathways—such as bone morphogenetic protein (BMP), Wnt/β-catenin, and the transcription factor Runx2—leading to the phenotypic transdifferentiation of vascular smooth muscle cells (VSMCs) into osteoblast-like cells[3, 4].

Eosinophils are regulated by the transcription factor GATA1 during hematopoiesis in the bone marrow and are classically recognized for their roles in allergic inflammation, particularly within the respiratory tract and lymphoid tissues[5–7]. Similar to other granulocytes, eosinophils secrete a range of cytokines, including interleukin-4 (IL-4) and IL-13, as well as chemokines and a distinctive repertoire of cationic granule proteins during inflammatory responses. These proteins include eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN).

Clinical observations have revealed a characteristic dynamic pattern of eosinophils in patients with myocardial infarction: both eosinophil counts and proportions decline markedly during the acute phase and subsequently rebound during the recovery phase following revascularization[8]. Consistent with these observations, studies by Tu et al. demonstrated that lower circulating eosinophil levels are associated with more severe myocardial injury and poorer clinical outcomes[9].

Mechanistic evidence further supports a direct pathogenic role of eosinophils in vascular remodeling. Guo et al. showed in murine models that eosinophils secrete mouse eosinophil-associated ribonuclease-1 (mEar1), which—together with its human homologues ECP and EDN—binds to bone morphogenetic protein receptors BMPR-1A and BMPR-1B[10]. This interaction activates the Smad1/5/8–Runx2 signaling axis, thereby promoting osteogenic transdifferentiation and calcification of vascular smooth muscle cells (VSMCs) in both mice and humans[11].

Collectively, these findings suggest a potential mechanistic link between eosinophil-derived cationic proteins and coronary artery calcification (CAC). However, clinical evidence directly addressing the association between circulating ECP levels and CAC in patients with coronary heart disease (CHD)

remains limited. Therefore, the present study aimed to investigate: (1) circulating ECP levels in patients with CHD; and (2) the associations between ECP levels, the severity of CAC, and the incidence of cardiovascular events (CVE)

Materials and Methods

Study Subjects

A total of 86 patients with coronary heart disease (CHD) and 65 healthy controls were recruited from the Department of Cardiology or Health Check-up Center of the Second Affiliated Hospital of Anhui Medical University between September 2024 and May 2025, with the diagnostic criteria for CHD patients consistent with the definition of stable angina pectoris specified in Internal Medicine; the inclusion criterion for CHD patients was that they must be aged over 18 years old and under 75 years old, while the exclusion criteria included severe abnormal liver and kidney function, allergic diseases, recent infections with various pathogens, diseases such as hematological diseases and malignant tumors, and recent administration of hormonal drugs and other relevant medications.

Standard Protocol Approvals and Patient Consents

All human studies described in this manuscript were conducted in accordance with national laws and the Declaration of Helsinki (1975, in its currently revised version). This study was approved by the Ethics Committee of the Second Affiliated Hospital of Anhui Medical University (No. : YJ-YX2022-004), and all participants provided written informed consent for their participation in this study.

Determination of Biochemical Parameters and ECP Concentration

Demographic characteristics such as age, gender, smoking history, diseases, complications, and the etiology of cardiovascular events (CVE) were collected from medical records. Venous blood samples were collected after an 8-hour fast the night before. Biochemical parameters included serum creatinine (Scr), hemoglobin (Hb), blood urea nitrogen (BUN), total cholesterol (TC), high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG), fasting blood glucose, calcium, phosphorus, albumin (ALB), and alkaline phosphatase (ALP), which were measured using an automatic biochemical analyzer (Beckman Coulter, AU5800) or immunoturbidimetry (Sysmex, XN9600). In addition, the estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI formula. NT-proBNP was detected using a fully automatic chemiluminescence immunoassay analyzer. The ECP protein level was measured by enzyme-linked immunosorbent assay (ELISA) (Cat. No.: JL17311, Jianglai Biotechnology Co., Ltd., Shanghai, China). Interleukin 3 (IL-3) and interleukin 14 (IL-14) were also measured by ELISA (Cat. No.: JL19252, JL19287, Jianglai Biotechnology Co., Ltd., Shanghai, China).

Evaluation of Coronary Artery Calcification (CAC)

CAC evaluation was performed using a multi-detector spiral computed tomography (MDCT) scanner (LightSpeed Plus; Philips iCT Brilliance 256, Koninklijke Philips N.V., Amsterdam, the Netherlands). Spiral CT images were transferred to a dedicated workstation and reviewed and scored by a technologist and a radiologist, who were blinded to the patients' clinical status and previous examination results. The left main coronary artery, left anterior descending artery, circumflex artery, and right coronary artery were evaluated for calcification scoring.The score for a single region was calculated by summing the areas with a density of ≥ 130 Hounsfield Units (HU) and multiplying the total area by a density-weighted factor based on the maximum density value within that region: a weight of 1 was assigned to regions with a density between 130 and 199 HU; a weight of 2 for 200 to 299 HU; a weight of 3 for 300 to 399 HU; and a weight of 4 for regions with a density of ≥ 400 HU. The total CAC score for each subject was the sum of the scores of all affected coronary arteries.Patients were categorized into different groups according to the calcification scores reported in previous studies: non-calcification and calcification groups. The calcification groups were further stratified as follows: (1) mild calcification: 0 to 100 points; (2) moderate calcification: 101 to 400 points; and (3) severe calcification: >400 points.

Data Analysis

Quantitative data were described as mean ± standard deviation if they followed a normal distribution; otherwise, median and interquartile range were used. One-way analysis of variance (ANOVA) or Kruskal-Wallis test was employed to compare the differences in anthropometric/biochemical indicators and ECP levels among multiple groups, and Bonferroni multiple significance test was used for post-hoc pairwise comparisons. Student's t-test or Mann-Whitney U test was used for comparing variables between two groups. Chi-square test or Fisher's exact test was applied for the analysis of categorical variables. Pearson or Spearman correlation analysis was used to infer the association between CAC scores and clinical/laboratory parameters.To identify the influencing factors of ECP levels and anthropometric/biochemical parameters on CAC scores, multiple linear regression (MLR) analysis was performed. Cox proportional hazards model and Kaplan-Meier curves were constructed to evaluate the risk factors that may lead to future cardiovascular events (CVE). Statistical analyses were conducted using R software (https://www.r-project.org/). All two-tailed P-values < 0.05 were considered statistically significant.

Results

Baseline Characteristics of the Study Subjects

A total of 151 subjects were enrolled in this study, including 86 patients with coronary heart disease (CHD) and 65 healthy controls. There was a statistically significant difference in age between CHD patients and healthy controls, indicating a higher incidence of CHD in the elderly. In addition, we found that compared with the healthy control group, CHD patients had significantly higher ECP levels ($P < 0.05$), while there were no significant differences in interleukin-4 (IL-4) and interleukin-13 (IL-13) levels between the two groups ($P > 0.05$) (Table 1).

Table 1
Comparison of Clinical Parameters between CAC Patients and the Control Group

Variables	Experimental Group	Control Group	p
Age (years)	63.45 ± 9.93	53.63 ± 16.77	0.001*
Female (n, %)	20(23%)	23(35%)	0.102
ECP(pg/ml)	11.09 (8.50,29.58)	3.61(2.92,5.82)	0.001*
IL-4(pg/ml)	237.43(201.31, 270.98)	234.6(218.62,270.16)	0.615
IL-13(pg/ml)	27.61(19.01, 34.81)	30.25(26.35, 33.43)	0.087

Abbreviations: *ECP, eosinophil cationic protein; IL-4, interleukin-4; IL-13, interleukin-13. *P < 0.05. Student's t-test or Mann-Whitney U test was used to compare age, ECP, IL-4, and IL-13 between the two groups, and Chi-square test was used to compare the distribution of females.*

Comparison of ECP Protein Expression Levels and Biochemical Indicators between Patients with Coronary Artery Calcification and Healthy Controls

According to the results of coronary artery CT scan, the enrolled subjects were divided into the CAC group (n = 86) and the non-CAC group (n = 65). As shown in Table 2, compared with the non-calcification group, patients in the CAC group were older, had higher proportions of diabetes mellitus, hypertension, and hyperlipidemia, and had significantly higher ECP protein expression levels (all P < 0.05). The levels of albumin (ALB) and high-density lipoprotein (HDL) in the non-calcification group were significantly higher than those in the CAC group (all P < 0.05). However, the smoking rate, blood urea nitrogen (BUN), low-density lipoprotein (LDL), blood glucose (Glu), and brain natriuretic peptide (BNP) in the non-calcification group were all lower than those in the CAC group (all P < 0.05). At the same time, we also found that there were no significant differences in the expression levels of IL-4 and IL-13 between the two groups (P > 0.05).

Table 2
Comparison of Demographic and Clinical Characteristics between the CAC Group and the Non-CAC Group

Variables	CAC group(n = 86)	non-CAC group(n = 65)	p
Age (years)	67(57,71)	58(40,67)	0.001*
Hypertension (n%)	67(78%)	23(35%)	0.001*
Diabetes mellitus (n%)	31(36%)	12(18%)	0.036*
Smoke(n%)	53(61%)	23(35%)	0.001*
ECP(pg/ml)	0.1(0.05, 0.175)	0.05(0.02, 0.18)	0.053
Scr(umol/L)	83.81 ± 80.98	52.29 ± 14.47	0.002*
eGFR (ml/min*1.73m2)	97.74 ± 44.8	113.77 ± 21.77	0.008*
BUN (mmol/L)	6.83 ± 2.33	4.76 ± 1.22	0.001*
ALP(U/L)	74.81 ± 17.56	85.20 ± 27.64	0.005
ALB(g/l)	40.45(38.27,43.72)	42.16(40.55,45.25)	0.001*
TC (mmol/L)	43.16 ± 3.43	41.29 ± 5.02	0.001*
LDL (mmol/L)	2.23(1.79, 3.26)	1.29(0.96, 2.54)	0.001*
HDL(mmol/L)	1.11(0.9,1.36)	2.35(1.93,2.91)	0.001*
TG(mmol/L)	1.21(0.92,1.79)	1.20(1.02,1.49)	0.516
Glu(mmol/L)	6.52 ± 3.07	4.53 ± 0.46	0.001*
Ca(mmol/L)	2.27(2.20,2.34)	2.33(2.28,2.41)	0.001*
P(mmol/L)	1.09(0.96,1.23)	1.36(1.19,1.51)	0.001*
BNP(ng/L)	99(34, 194)	72(31, 129)	0.048*
Hb(g/L)	138.5 ± 18.5	131.9 ± 16	0.880
ECP(pg/ml)	11.09(8.50,29.58)	3.16(2.24,3.65)	0.001*
IL-4(pg/ml)	237.43(201.31,270.98)	234.63(218.62,270.16)	0.615
IL-13(pg/ml)	27.61(19.01,34.81)	30.25(26.35,33.43)	0.087

Abbreviations: Scr, serum creatinine; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; ALP, alkaline phosphatase; ALB, albumin; TC, total cholesterol; TG, triglyceride; Glu, glucose; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Ca, calcium; P, phosphorus; BNP, brain natriuretic peptide; Hb, hemoglobin; IL-4, interleukin-4; IL-13, interleukin-13. Student's t-test or Mann-Whitney U test was used for comparing variables between the two groups, and Chi-square test or Fisher's exact test was applied for the analysis of categorical variables.

Table 3
Correlation between Clinical and Laboratory Parameters and CAC Score

Variables	r	P
Age	0.315	0.003*
ECP(pg/ml)	0.934	0.001*
IL-4(pg/ml)	0.051	0.639
IL-13(pg/ml)	0.029	0.794
HB(g/L)	0.1	0.359
Scr(umol/L)	0.151	0.166
eGFR (ml/min*1.73m2)	0.198	0.068
BUN (mmol/L)	0.224	0.038*
ALP(U/l)	0.095	0.386
ALB(g/l)	0.023	0.830
TC (mmol/L)	-0.194	0.073
LDL (mmol/L)	− .051	0.064
Glu(mmol/L)	0.382	0.725
BNP(ng/L)	0.228	0.034*
ECP(pg/ml)	0.039	0.721

Abbreviations: Age, age; ECP, eosinophil cationic protein; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; ALP, alkaline phosphatase; ALB, albumin; TC, total cholesterol; TG, triglycerides; Glu, glucose; LDL, low-density lipoprotein; BNP, brain natriuretic peptide; HB, hemoglobin; IL-4, interleukin-4; IL-13, interleukin-13. Spearman correlation analysis was used to infer the relationship between CAC score and clinical/laboratory parameters.

Comparison of ECP Protein Expression Levels in Patients with Different Degrees of CAC

To investigate the relationship between the severity of coronary artery calcification (CAC) and eosinophil cationic protein (ECP) levels, all enrolled subjects were divided into five groups according to their coronary artery calcium score (CACS),including the mild group (n = 43), moderate group (n = 20), severe group (n = 23), and non-calcified group (n = 65). Further analysis showed that with the increase in the severity of coronary artery calcification, the expression level of ECP protein also increased significantly. The ECP expression level in patients with very severe coronary artery calcification was significantly higher than that in patients with mild CAC and non-CAC subjects (all $P < 0.05$). (Fig. 1)

Correlation between Clinical and Laboratory Parameters and CAC Score

To explore the correlation between coronary artery calcium (CAC) score and multiple clinical and laboratory parameters in patients with chronic kidney disease (CKD), Spearman correlation analysis was performed. The results showed that age, eosinophil cationic protein (ECP) expression level, blood urea nitrogen (BUN) level, and brain natriuretic peptide (BNP) level were all positively correlated with the severity of calcification. However, previous studies and literatures have indicated that high-density lipoprotein (HDL) is negatively correlated with coronary artery calcification, while low-density lipoprotein (LDL) and triglycerides (TG) are positively correlated with coronary artery calcification, which was not observed in this study. It is considered that the patients included in this study had taken lipid-lowering drugs, and the impact of drug administration on the results of this study cannot be excluded.

Identification of Potential Influencing Factors of CAC Score by Multiple Linear Regression (MLR)

The independence of the correlation between CAC score and several clinical and laboratory indicators was evaluated by performing stepwise MLR, where CAC score was defined as the dependent variable. ECP protein expression level and indicators that were significantly correlated with CAC score in previous studies were used as independent variables in the MLR model. MLR analysis showed (see Table 4) that ECP protein expression level, age, BNP level, and BUN were significant influencing factors of CAC score.

Table 4
Multiple Linear Regression Analysis of Different Predictors on CAC Score

Model	Independent Variables	R	R^2	Adjusted R^2	F	P	Standard Error of Estimate	Durbin-Watson Value
1	CAC score	0.934	0.872	0.870	569.914	0.001*	113.575	0.383
	ECP					0.001		
2	CAC score	0.934	0.872	0.868	281.567	0.001*	114.256	-
	ECP					0.001		
	Age					0.980		
3	CAC score	0.934	0.872	0.867	186.196	0.001*	114.750	-
	ECP					0.001		
	Age					0.957		
	BUN					0.593		
4	CAC score	0.945	0.894	0.888	160.138	0.001*	105.86	-
	ECP					0.001		
	Age					0.860		
	BUN					0.143		
	BNP					0.025		

Abbreviations: CAC, coronary artery calcium; ECP, eosinophil cationic protein; BUN, blood urea nitrogen; BNP, brain natriuretic peptide; Age, age. Multiple linear regression (MLR) analysis was used to determine the influencing factors of ECP level and anthropometric/biochemical parameters on CAC score.

Cox Regression for Identifying Risk Factors of Cardiovascular Events in Patients with Coronary Artery Calcification (CAC)

Eighty-six patients with coronary artery calcification were followed up routinely, and the occurrence of cardiovascular events (CVE) was observed by telephone. A total of 39 cases of CVE were reported during the follow-up period, including 3 cases of heart failure, 3 cases of myocardial infarction, 5 cases of arrhythmia, 10 cases of cerebral infarction, 1 case of cerebral hemorrhage, and 17 cases of angina pectoris. To explore the risk factors of CVE in patients with coronary artery calcification, univariate and multivariate Cox regression analyses were performed. As shown in Table 5, univariate Cox regression analysis indicated that CAC score, ECP, BNP, and hypertension were associated with a high risk of CVE. However, the results of multivariate Cox regression analysis demonstrated that only hypertension was correlated with the risk of CVE.

Table 5
Univariate Cox Regression Analysis of Risk Factors for CVE in CAC Patients

Variables	β	P	HR	95%CI
EOS	0.827	0.544	2.285	0.159, 32.938
CAC Score	0.001	0.016*	1.001	1.000,1.002
Female	0.853	0.057	2.347	0.975,5.649
ECP(pg/ml)	0.023	0.013	1.023	1.005,1.041
IL-4(pg/ml)	0.005	0.172	1.005	0.998,1.012
IL-13(pg/ml)	0.014	0.442	1.014	0.979.1.050
Hb(g/L)	0.007	0.445	1.007	0.989,1.025
Scr(μmol/L)	-0.001	0.542	0.999	0.994,1.003
eGFR (ml/min*1.73m2)	0.001	0.960	1	0.993,1.008
BUN(mmol/L)	0.028	0.668	1.028	0.906.1.167
ALP(U/l)	0.005	0.569	1.005	0.988,1.023
ALB(g/l)	-0.051	0.218	0.950	0.867,1.031
TC(mmol/L)	0.015	0.911	1.015	0.783,1.315
HDL(mmol/L)	-0.444	0.456	0.641	0.2, 2.061
LDL(mmol/L)	0.01	0.954	1.010	0.733, 1.39
TG(mmol/L)	-0.092	0.595	0.912	0.650, 1.28
Ca(mmol/L)	-1.744	0.245	0.175	0.009, 3.496
P(mmol/L)	0.123	0.859	1.131	0.290, 4.417
BNP(pg/ml)	0.001	0.036	1.000	1.000, 1.000
Smoke(n%)	-0.407	0.232	0.666	0.340,1.302
Hypertension(n%)	0.895	0.01	2.447	1.239,4.836
Diabetes mellitus (n%)	7.986	0.939	1.196	0.286,4.503

Abbreviations: Age, age; ECP, eosinophil cationic protein; Scr, serum creatinine; eGFR, estimated glomerular filtration rate; BUN, blood urea nitrogen; ALP, alkaline phosphatase; ALB, albumin; TC, total cholesterol; TG, triglycerides; Glu, glucose; LDL, low-density lipoprotein; BNP, brain natriuretic peptide; HB, hemoglobin; IL-4, interleukin-4; IL-13, interleukin-13. The Cox proportional hazards model was constructed to evaluate the possible risk factors that contributed to the future cardiovascular events (CVE)

To investigate the impact of eosinophil cationic protein (ECP) levels and the severity of coronary artery calcification (CAC) on cardiovascular adverse events (CVE), ECP levels were divided into two groups based on the median (11.09 pg/ml), and coronary artery calcification was divided into mild, moderate, and severe three groups according to the CAC score of 400. Kaplan-Meier analysis and log-rank test showed that the high ECP level group (> 11.09 pg/ml) and the severe CAC group had a higher risk of CVE (both $P < 0.001$) (Figs. 2 and 3).

Discussion

In this study, we systematically evaluated circulating eosinophil cationic protein (ECP) levels in patients with coronary artery calcification (CAC) and investigated their clinical significance. We found that serum ECP levels were significantly elevated in patients with coronary heart disease and in those with CT-confirmed CAC compared with non-calcified controls, and that ECP levels increased progressively with calcification severity as assessed by the coronary artery calcium score (CACS). ECP was positively correlated with CACS and remained independently associated with calcification burden after multivariable adjustment. Although higher ECP levels and more severe CAC were associated with increased cardiovascular event risk in Kaplan–Meier analysis, only hypertension remained an independent predictor in multivariate Cox regression. To our knowledge, this is the first study to comprehensively delineate the relationship between circulating ECP, CAC severity, and cardiovascular outcomes.

Substantial evidence indicate that eosinophils exert a dual role in the pathogenesis of cardiovascular diseases, encompassing both deleterious and protective effects[5–8]. On the one hand eosinophils can directly damage cardiomyocytes and vascular endothelium by releasing toxic granules such as cationic proteins and peroxidases, participate in the inflammatory progression of diseases such as myocarditis, cardiomyopathy, and atherosclerosis, and also promote myocardial fibrosis to exacerbate cardiac insufficiency[10, 12–14].On the other hand, they can play a protective role in vascular injury repair and maintenance of myocardial homeostasis by regulating the immune microenvironment, removing necrotic tissue, and inhibiting excessive inflammatory responses[10, 15, 16].

Our study results demonstrated that high ECP levels and severe CAC were associated with an increased risk of CVE[17], which suggests that ECP and CAC severity may be potential predictive indicators for CVE in patients with coronary artery calcification. The possible mechanism is that ECP, as a toxic granule released by eosinophils, can damage vascular endothelial cells, promote the formation of atherosclerotic plaques, and accelerate the progression of coronary artery calcification[13, 14]; meanwhile, severe coronary artery calcification indicates more severe atherosclerotic lesions, which are more likely to rupture or cause vascular stenosis, thereby inducing cardiovascular adverse events[18, 19].

The core focus indicator of this study was ECP protein. The results showed that the expression level of ECP in the calcification group was significantly higher than that in the non-calcification group, suggesting that high ECP protein expression is one of the common characteristics in the population with vascular

calcification[10]. In addition, this study also found that age, blood urea nitrogen (BUN), and brain natriuretic peptide (BNP) are also factors affecting the severity of coronary artery calcification[20–22]. It is worth noting that previous studies have shown that decreased glomerular filtration rate, elevated low-density lipoprotein, and elevated triglycerides are all important contributing factors to atherosclerosis, but this study did not obtain consistent results. It is speculated that this may be related to the small sample size and the fact that the included population has received drug treatment, and the interference of drugs on the study results cannot be excluded[23]. At the same time, we also found that severe CAC, high ECP expression, high BNP levels, and hypertension are risk factors for CVE in patients[17, 24, 25].

This study confirmed that ECP protein levels are significantly positively correlated with atherosclerosis, suggesting that ECP may be involved in the calcification process by acting directly on the vascular wall, rather than simply reflecting the systemic inflammatory state. This conclusion is consistent with the research results of Professor Guo Junli et al. Their study found that after transfusing exogenous eosinophil-derived ribonuclease 1 (EDN1)-donor eosinophils into ΔdblGATA mice (eosinophil-deficient mice), the degree of coronary artery calcification in mice was significantly aggravated. Finally, it was confirmed that eosinophils can release ECP protein and promote smooth muscle cell (SMC) calcification and atherosclerosis formation through the BMPR-1A/1B-Smad-1/5/8-Runx2 signaling pathway[18].

It should be emphasized that ECP, as a cationic protein derived from eosinophils, its expression level is affected by multiple factors such as infection, allergy, and inflammation. Before the occurrence of acute coronary events, most patients with coronary artery calcification are often in a non-inflammatory stage, and the release of cationic proteins by eosinophils depends on blood cell integrity. Therefore, the detection of whole blood samples can more truly reflect the physiological state of ECP in the body[26]. A study in Denmark has confirmed that blood eosinophil count is correlated with calcification scores of different arterial segments in the human body[10]. Combined with the result of this study that the expression level of ECP protein in the coronary artery calcification group was significantly higher than that in the healthy control group, it suggests that evaluating the expression level of ECP protein in the population with coronary artery calcification has important guiding significance for the clinical prevention and treatment of coronary artery calcification, and reducing the expression of ECP protein may effectively promote the prevention and control of coronary artery calcification.

Univariate Cox proportional hazards regression analysis showed that ECP levels were significantly associated with an increased risk of cardiovascular disease in patients with coronary artery calcification[27, 28]. The mechanism by which high ECP levels promote vascular calcification can be explained as follows: ECP protein can bind to bone morphogenetic protein receptors (BMPR-1A/1B), activate the Smad-1/5/8-Runx2 signaling pathway, induce the osteoblastic phenotypic transformation of SMCs, and promote the expression of collagen I, osteocalcin, and osteopontin, leading to vascular wall calcification. Calcified plaques are hard in texture, prone to rupture and induce thrombosis, thereby increasing the risk of acute coronary syndrome (ACS)[18, 29, 30]. Studies have shown that arterial calcification is significantly reduced in ΔdblGATA mice (eosinophil-deficient mice), while supplementation with eosinophils or mEar1 can restore the calcification phenotype, confirming the key

role of eosinophils in calcification. At the same time, the continuous activation of the BMPR-1A/1B-Smad-1/5/8-Runx2 pathway can not only promote calcification but also may exacerbate vascular wall mineralization by up-regulating the activity of alkaline phosphatase, forming a vicious cycle of "calcification-inflammation". As a severe stage of coronary atherosclerosis, the core of coronary calcification exacerbating heart failure is the comprehensive effect of multiple mechanisms and links, and it forms a significant synergistic effect with metabolic disorders in the cardio-endocrine cross-field, ultimately constructing a vicious cycle of "myocardial ischemia - cardiac function impairment - heart failure progression"[31, 32]. Similarly, in our study, BNP, CAC score, and hypertension were also associated with a high risk of cardiovascular disease. Compared with traditional calcification-related markers such as osteocalcin and osteopontin, ECP may earlier indicate the driving mechanism of calcification, rather than simply reflecting the degree of calcification[3].

This study was a prospective cohort study aimed at verifying the predictive value of ECP for the progression of coronary artery calcification. However, this study had certain limitations: some participants in the included population had received drug treatments such as antiplatelet agents and angiotensin-converting enzyme inhibitors (ACEIs)[33–35]. Such drugs may affect the process of vascular calcification by inhibiting inflammatory responses, regulating the vascular microenvironment, and other pathways, thereby interfering with the study results; in addition, there is currently no unified standard for ECP detection, and its plasma levels are affected by various factors such as the activation state of eosinophils and sample processing procedures. In the future, it is necessary to establish a stable and standardized ECP detection method, clarify its normal reference range and cut-off value, and lay a foundation for its clinical transformation.

Conclusion

As a specific marker of eosinophil activation, ECP is not only closely associated with coronary artery calcification but also may directly participate in the process of vascular calcification through BMPR-mediated molecular mechanisms. Although the existing evidence still needs further verification, ECP is expected to become a novel biomarker for risk assessment of coronary artery calcification and provide a potential target for the targeted therapy of coronary artery calcification. Future studies should focus on mechanism verification, clinical transformation, and the development of intervention strategies to achieve precision stratification and individualized management of coronary heart disease risk.

Abbreviations

ACEIs
Angiotensin-converting enzyme inhibitors
ACS
Acute coronary syndrome
ALB
Albumin

ALP
Alkaline phosphatase
BMP
Bone morphogenetic protein
BNP
Brain natriuretic peptide
CAD
Coronary artery disease
CAC
Coronary artery calcification
CACS
Coronary artery calcium score
CVE
Cardiovascular events
EDN
Eosinophil-derived neurotoxin
ECP
Eosinophil cationic protein
SMC
Smooth muscle cell
VC
Vascular calcification
VSMCs
Vascular smooth muscle cells

Declarations

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Authors 'contributions:

Feng Gao participated in study design and manuscrpt draft. Tian Rui are contribute to the performance of research and data collection. Guangquan Hu,Jie Wu,Baoqian Zhang,Bicong Gao and Haicheng Xu participated in the data collection. Jiqing Ye ingyuan Liu and Danfeng Zhang are contributed to study design and perfomance of research. Feng Gao is the senior author of this manuscript and contributed to the study design ,implementation and manuscrpt draft.All authors reviewed the article and approved the submitted publication.

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Data availability

Due to ethical considerations and participant confidentiality, requests for access to the raw data should be directed to the corresponding author.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Anhui Medical University (No.: YJ-YX2022-004), and all participants provided written informed consent for their participation in this study.

Consent for publication

Not applicable.

Clinical trial number:

Not applicable.

Competing interests

The authors declare no competing interests.

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Figures

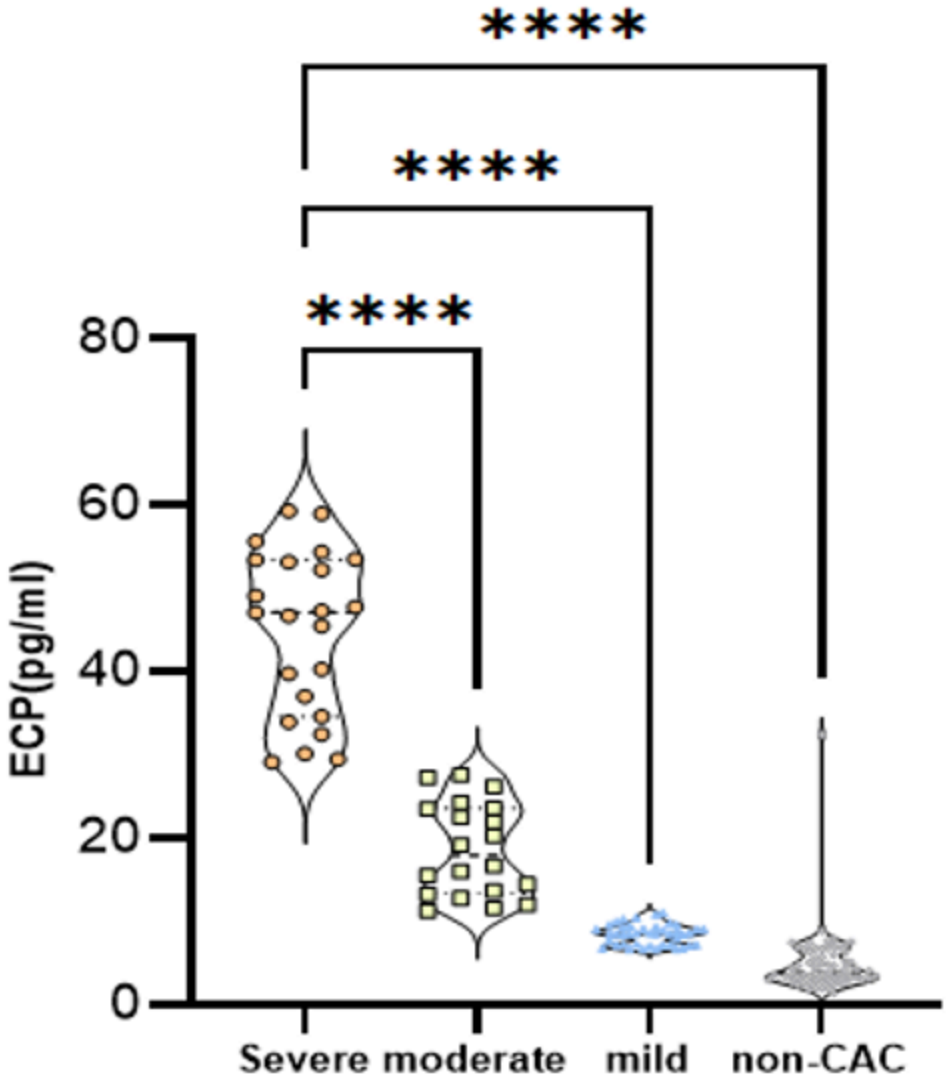


Figure 1

Differences in ECP levels between mild, moderate, severe CAC groups and non-CAC group (ns: $P < 0.05$, *$P < 0.05$). Abbreviation: CAC, coronary artery calcification; One-way analysis of variance (ANOVA) was used to compare the differences in ECP levels among multiple groups; Bonferroni multiple significance test was used for correction in inter-group comparisons.

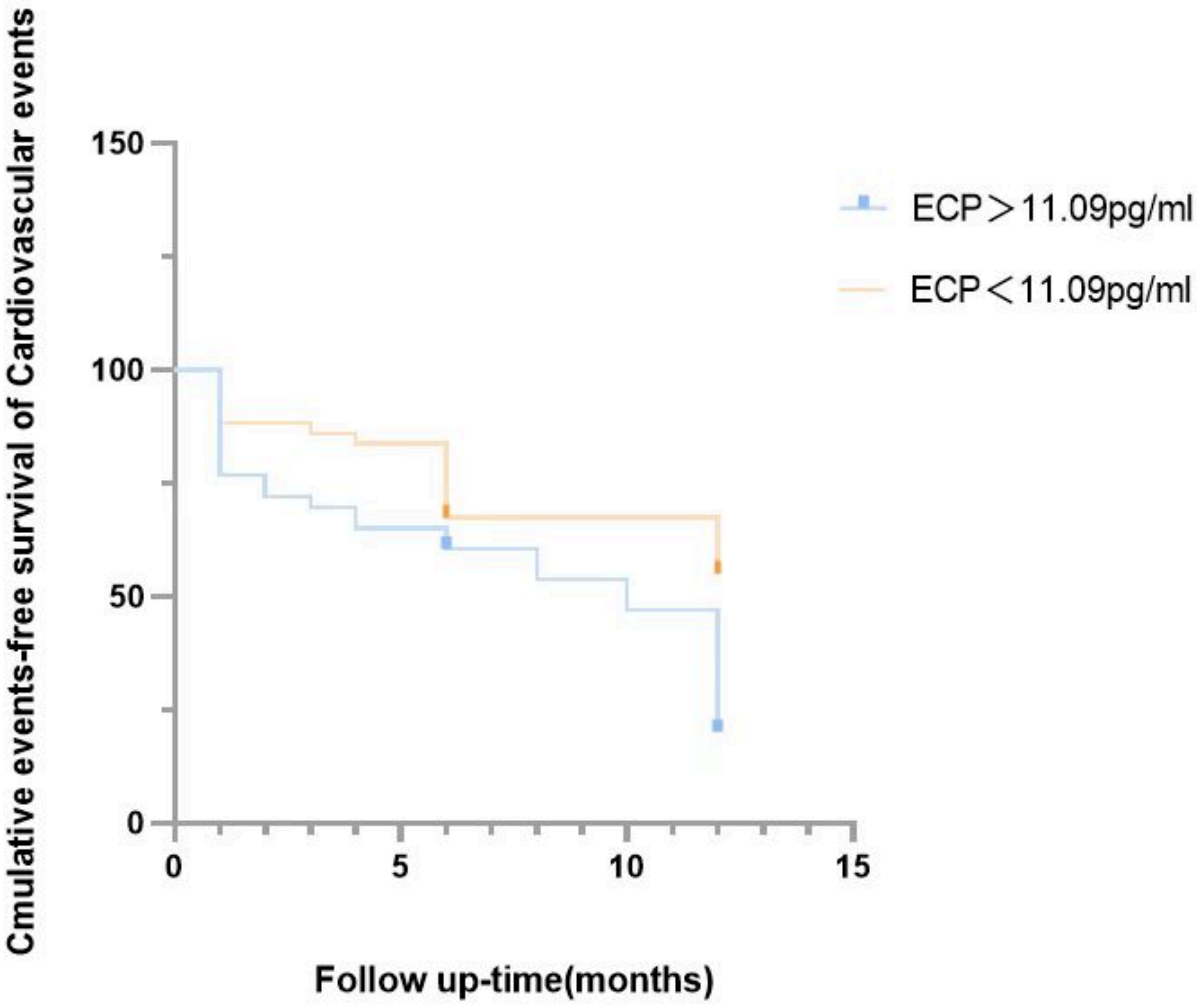


Figure 2

Kaplan-Meier curve of ECP levels for non-fatal cardiovascular events. The group with ECP level above the median (ECP ≥ 11.09 pg/ml) and the group with ECP level below the median (ECP < 11.09 pg/ml) (log-rank test, P < 0.05). The cumulative event-free survival rate of CVE was analyzed by Kaplan-Meier survival curve and log-rank test for statistical analysis.

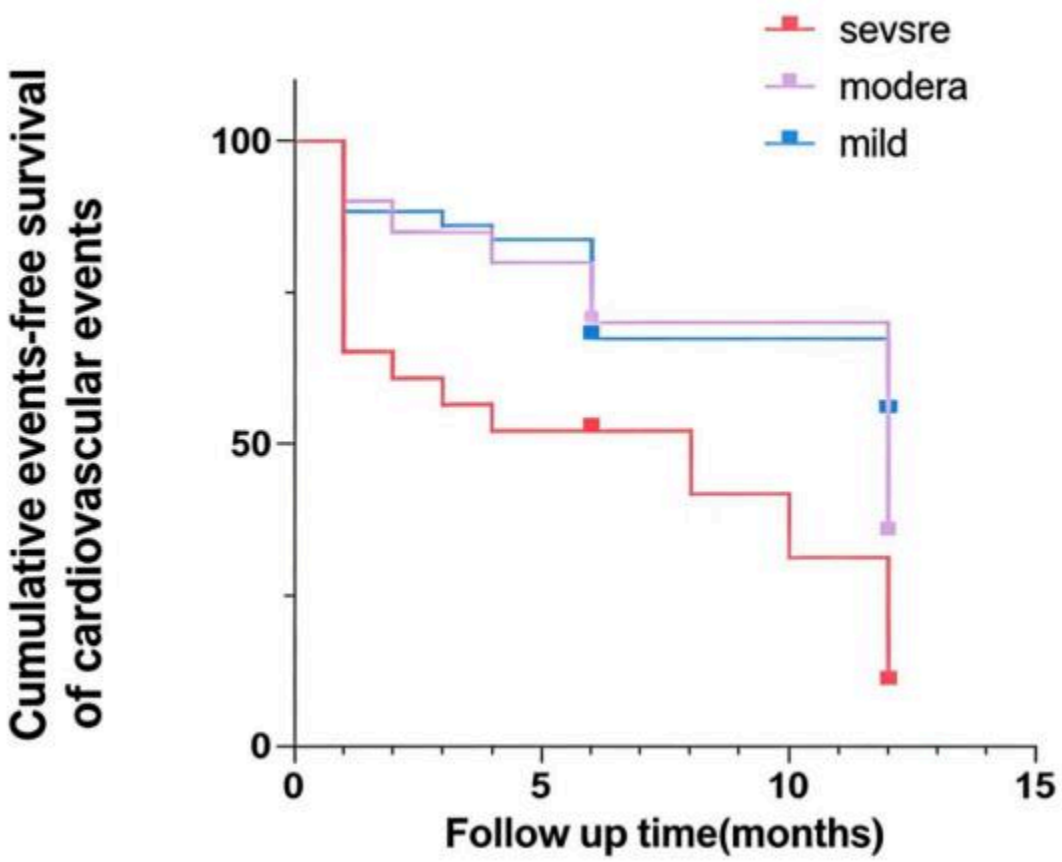


Figure 3

Kaplan-Meier curve of non-fatal cardiovascular events based on coronary artery calcification; the severe CAC group (CACs ≥ 400), the moderate CAC group (100 < CACs < 400), and the mild CAC group (CACs < 100) (log-rank test, P < 0.001). The cumulative event-free survival rate of CVE was analyzed using the Kaplan-Meier survival curve, with statistical analysis performed by the log-rank test.