Atherosclerosis 286 (2019) 128–134

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Eosinophils count in peripheral circulation is associated with coronary artery disease

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HIGHLIGHTS

- A total of 5287 patients who underwent coronary angiography were included in the study.
- The eosinophil count may be strongly negativly related to the subtype and severity of CAD.
- Eosinophils may be a promising biomarker that can accurately and independently predict CAD severity and myocardial infarction.

ARTICLE INFO

Keywords:
Eosinophils
Coronary artery disease
Gensini score
Risk stratification

ABSTRACT

Background and aims: Allergic asthma can accelerate atherosclerosis, a disease in which plaque is deposited onto arterial walls and that may lead to coronary artery disease (CAD). Eosinophils are the most important effector cells in allergic asthma and are likely to become novel biomarkers for risk stratification of patients with CAD, but the relationship between eosinophil count and CAD remains unclear. We aimed to evaluate this relationship and the use of eosinophils in predicting CAD.

Methods: A total of 5287 patients who underwent coronary angiography were recruited. Their biochemical parameters, including eosinophil count, were measured and their correlation with the severity of coronary artery stenosis, as quantified by the Gensini score system, was evaluated.

Results: The percentages of eosinophils in leukocytes (PELs) were lower in CAD patients ($p < 0.001$), and had a significant negative correlation with Gensini scores ($r = -0.112$, $p < 0.001$). PELs were also significantly lower in acute myocardial infarction patients ($p < 0.001$). After adjusting for baseline differences, low PELs remained strongly associated with severe CAD and acute coronary arterial thrombotic event. Receiver-operating characteristic curve analysis showed that combining PELs with traditional risk factors in predictive models for CAD severity ($z = 4.470$, $p < 0.001$) or acute coronary arterial thrombotic event ($z = 9.435$, $p < 0.001$) improved the predictive capabilities of those models.

Conclusions: PELs, at least in patients undergoing coronary angiography, may be strongly related to the subtype and severity of CAD and, therefore, eosinophil count may be an accurate and independent biomarker to predict CAD severity and acute coronary arterial thrombotic events.

1. Introduction

Allergic asthma and atherosclerosis, a disease in which arteries are narrowed due to plaque deposition, leading to the possibility of coronary artery disease (CAD), are chronic inflammatory diseases with potential systemic impacts. Atherosclerotic lesions and asthmatic

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https://doi.org/10.1016/j.atherosclerosis.2019.05.027
Received 14 January 2019; Received in revised form 24 April 2019; Accepted 26 May 2019
Available online 28 May 2019
0021-9150/

bronchioles and alveoli are rich in similar types of inflammatory cells, suggesting that allergic asthma and atherosclerosis may share similar pathogenesis processes and interact with each other [1]. Recently, research has shown that allergic lung inflammation promotes atherogenesis and aortic lesion formation, and asthma severity correlates with the degree of pathological vessel changes in atherosclerosis [2]. Our previous study also found that allergic asthma accelerates atherosclerosis by modulating the balance of effector and regulatory T cells [3]. Moreover, clinical studies have shown that high serum Immunoglobulin E levels, a key component of allergic asthma, were significantly increased in patients with unstable plaque [4] and associated with CAD severity [5]. Similarly, patients with allergic asthma also show a high risk of developing atherosclerosis [6].

Eosinophils are pleiotropic multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes and are one of the most important effector cells in allergic asthma progression [7]. So, due to the possible connection between allergic asthma and atherosclerosis, it is important to know whether eosinophils also promote the development of atherosclerosis and, in turn, CAD. Although there are few eosinophils present in atherosclerotic plaque, studies have shown that eosinophils may promote plaque progression in coronary arteries, leading to cardiovascular events via the release of eosinophils' mediators [7], and are involved in several steps leading to plaque progression and acute thrombotic events [8]. Clinical studies have also found that high eosinophil levels are associated with increased mortality rates [9]. In an autopsy study assessing patients who died of acute myocardial infarctions, the eosinophil count in the inflammatory response was significantly higher in hearts that suffered cardiac ruptures than in those without cardiac ruptures [10]. Opposite to this, another study reported that low eosinophil percentages suggest serious myocardial damage in patients with acute coronary syndrome [11] and are associated with the increased short-term incidence of heart failure and coronary death [12]. However, there are still some studies showing high eosinophil levels are not independently associated with the prevalence and severity of CAD [8,13]. Additionally, eosinophil cationic protein (ECP) levels, the biomarker of eosinophil activation, are significantly higher in acute coronary syndrome cases and can independently predict CAD severity [14,15]. ECPs are also associated with atherosclerotic burden and poor prognoses in patients undergoing stent implantation [14,16]. Therefore, considering these conflicting studies, the direct relationship between eosinophils and CAD remains uncertain.

In this present study, we aimed to evaluate the relationship between the amount of eosinophils in peripheral circulation and various CAD subtypes and severities, and the ability of eosinophils to predict severe CAD and acute coronary arterial thrombotic event.

2. Patients and methods

2.1. Study population

We recruited 5287 patients who underwent coronary angiography at Xi'an Jiaotong University's First Affiliated Hospital of Medical College from April 2017 to May 2018. To limit potential confounding factors, patients were excluded when they had: allergic asthma, autoimmune diseases, a history of allergic diseases, parasitic infections, current infections, malignancies, severe liver or kidney dysfunction, heart failure or shock, rheumatic heart diseases, and valvular heart diseases. Patients who underwent coronary artery bypass surgery were also not included in this study considering graft complexity. This study was approved by the Ethics Committee of Xi'an Jiaotong University and was performed in accordance with the Declaration of Helsinki. All study participants provided written informed consent.

Patients were first categorised by whether they had CAD. CAD was defined as having > 50% stenosis in ≥1 major coronary artery. CAD patients were then further divided into four groups; stable angina pectoris (SAP), unstable angina pectoris (UAP), non-ST-elevation myocardial infarction (NSTEMI), and acute ST-elevation myocardial infarction (ASTEMI) groups. The acute coronary arterial thrombotic event refers to the NSTEMI/ASTEMI. Moreover, to further clarify the correlation between PELs and the severity of CAD, patients were also divided into three groups according to PEL tertiles (≤1.00; 1.01–2.00; > 2.01%).

2.2. Patient characteristics and biochemical measurements

Patients' baseline characteristics and clinical data, including age, gender, hypertension, diabetes, family history of CAD, and smoking status, were recorded from patients' standard medical records. Current smokers were defined as patients who still smoking tobacco products within three months prior to hospital admission. Traditional cardiovascular risk factors include age, gender, smoking status, family history of CAD, hypertension, diabetes and lipid profiles.

At one-day post-admission, after a 12-h overnight fast, peripheral venous blood samples were drawn into K_2EDTA-anticoagulated standardized tubes (BD Biosciences, USA) and samples' absolute eosinophil count (AEC) were measured with an automatic haematology analyser (Sysmex 2100, Japan) within 30 min after blood collection. PELs were then calculated as the percentages of eosinophil in leukocytes of peripheral circulation. Lipid profiles and other biochemical parameters were measured using standardized methods in laboratory.

2.3. Coronary angiography and assessing CAD severity

Coronary angiography was performed by experienced interventional cardiologists who utilized the femoral or radial artery approach with a conventional angiography unit. Coronary artery stenosis was assessed using multiple projected images. Two interventional cardiologists with at least five years of experience independently evaluated coronary atherosclerotic lesion severity. The Gensini score system, a quantitative scoring tool that can objectively evaluate coronary arterial stenosis, was used to quantify the severity of coronary artery stenosis (Gensini scores, 1 = 1–25% stenosis; 2 = 26–50% stenosis; 4 = 51–75% stenosis; 8 = 76–90% stenosis, 16 = 91–99% stenosis, and 32 = 100%) [17]. Only the most stenotic segments were used to evaluate stenosis severity for each patient. Then the stenosis score multiplied the coefficient, which is given for each main coronary artery and each segment, and each patient's total score was the sum of the scores for every branch.

For patients who had previously undergone percutaneous coronary intervention (PCI), the Gensini scores were the sum of restenosis scores and other coronary artery stenosis scores if restenosis was observed. Treated blood vessels were not scored if restenosis was not observed.

The severe CAD was defined as the top Gensini score tertile.

2.4. Statistical analyses

Data were statistically analysed using SPSS 20.0 and MedCalc 18.2 software. Continuous variables were expressed as "mean ± standard deviation" and categorical variables were expressed as count and corresponding percentage. All continuous data were analysed for normality using the Shapiro-Wilk normality test. One-way analysis of variance was used to determine the statistical significance of the differences among the groups if the values for their corresponding parameters followed a normal distribution. Otherwise, the Mann-Whitney U and Kruskal-Wallis H tests were used. Chi-square tests and Fisher's exact test were used to compare the statistical significance of categorical variables between each group. After correcting for baseline confounding factors, a multivariate logistic regression analysis was used to evaluate the relationship between AECs and CAD severity, and the relationship between PELs and CAD severity in a stepwise forward conditional manner. Area under the receiver-operating characteristics

Table 1
Baseline characteristics in patients with or without CAD.

	Non-CAD group (N = 1080)	CAD group (N = 4207)				*p* value
		SAP (N = 286)	UAP (N = 2912)	NSTEMI (N = 247)	ASTEMI (N = 762)	
Age(yr)	59.51 ± 10.07	62.63 ± 10.04	61.99 ± 9.60	61.11 ± 11.22	58.67 ± 11.62	< 0.001
Male, n (%)	551 (51.0)	199 (69.6)	2041 (70.1)	182 (73.7)	612 (80.3)	< 0.001
Hypertension n (%)	522 (48.3)	169 (59.1)	1761 (60.5)	153 (61.9)	392 (51.4)	< 0.001
DM, n (%)	247 (22.9)	98 (34.3)	977 (33.6)	82 (33.2)	280 (36.7)	< 0.001
Current smoker, n (%)	355 (32.9)	134 (46.9)	1452 (49.9)	144 (58.3)	463 (60.8)	< 0.001
Family history, n (%)	165 (15.3)	43 (15.0)	499 (17.1)	53 (21.5)	249 (32.7)	< 0.001
Previous PCI, n(%)	4 (0.4)	56 (19.6)	927 (31.8)	82 (33.2)	202 (26.5)	< 0.001
Gensini score	4.35 ± 3.31	24.61 ± 22.61	37.29 ± 31.38	51.34 ± 34.31	49.08 ± 32.67	< 0.001
Biochemical parameters						
Total cholesterol, mmol/L	3.88 ± 0.92	3.77 ± 0.99	3.70 ± 0.98	3.97 ± 0.99	4.03 ± 0.95	< 0.001
LDL-C, mmol/L	2.22 ± 0.78	2.18 ± 0.86	2.11 ± 0.82	2.39 ± 0.82	2.46 ± 0.80	< 0.001
HDL-C, mmol/L	1.04 ± 0.27	1.01 ± 0.26	0.96 ± 0.23	0.94 ± 0.23	0.93 ± 0.21	< 0.001
Triglycerides, mmol/L	1.52 ± 1.24	1.51 ± 1.06	1.53 ± 1.00	1.71 ± 1.50	1.67 ± 1.07	0.001
HbA1C, %	5.52 ± 1.29	5.78 ± 1.71	5.88 ± 1.82	6.07 ± 1.34	6.16 ± 1.52	< 0.001
Creatinine, μmol/L	61.07 ± 16.11	70.33 ± 57.71	65.83 ± 27.97	69.48 ± 30.46	66.67 ± 19.74	< 0.001
Uric acid, μmol/L	305.13 ± 80.47	318.94 ± 83.92	317.79 ± 82.14	316.14 ± 82.87	322.34 ± 90.07	< 0.001
HCY, μmol/L	18.42 ± 11.18	19.28 ± 9.37	20.43 ± 12.85	21.21 ± 12.39	23.49 ± 16.99	< 0.001
hs-CRP, mg/L	1.73 ± 2.37	2.18 ± 2.94	2.31 ± 2.80	4.47 ± 3.71	4.77 ± 3.74	< 0.001
apoA1, g/L	1.17 ± 0.20	1.13 ± 0.20	1.11 ± 1.85	1.06 ± 0.17	1.04 ± 0.17	< 0.001
Leukocyte, 10^9/L	6.29 ± 2.31	6.44 ± 1.74	6.76 ± 1.98	8.02 ± 2.22*,#	9.16 ± 3.15*,#	< 0.001
AEC, 10^9/L	0.12 ± 0.11	0.13 ± 0.12	0.13 ± 0.11	0.11 ± 0.11*,#	0.09 ± 0.09*,#	< 0.001
PELs, %	2.00 ± 1.84	2.03 ± 1.78	1.96 ± 1.58	1.48 ± 1.64*,#	1.10 ± 1.17*,#	< 0.001
Concomitant therapy						
Aspirin, n (%)	1041 (96.4)	263 (92)	2832 (97.3)	244 (98.8)	742 (97.4)	< 0.001
Clopidogrel/Ticagrelor, n (%)	1035 (95.8)	255 (89.2)	2837 (97.4)	243 (98.4)	743 (97.5)	< 0.001
Beta blockers, n (%)	788 (73)	215 (75.2)	2330 (80)	209 (84.6)	633 (83.1)	< 0.001
Statins, n (%)	1027 (95.1)	271 (94.8)	2835 (97.4)	243 (98.4)	743 (97.5)	< 0.001
ACEI/ARB, n (%)	755 (69.9)	209 (73.1)	2426 (83.3)	211 (85.4)	611 (80.2)	< 0.001

DM, diabetes mellitus; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HbA1C, glycated hemoglobin; HCY, homocysteine; hs-CRP, high sensitive C reactive protein; apoA1, apolipoprotein A1; AEC, absolute eosinophil count; PELs, percentages of eosinophils in leukocytes; AEC, absolute eosinophils count; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker.
*$p < 0.05$ *vs.* non-CAD/SAP; #$p < 0.05$ *vs.* UAP.

(ROC) curve analyses were used to evaluate the ability of different models in predicting severe CAD. The nonparametric approach of DeLong et al. [18] (MedCalc) was used to analyse differences between receiver-operating characteristics curves. All *p*-values were two-sided and a *p*-value of < 0.05 was considered significant.

3. Results

3.1. Study population baseline characteristics

A total of 3585 men and 1702 women (mean age, 61.00 ± 10.20 years) were included. The baseline characteristics of all patients are listed in Table 1. Coronary angiography revealed 1080 patients without CAD and 4207 patients with CAD (SAP group, 286; UAP group, 2912; NSTEMI group, 247; ASTEMI group, 762). We observed that the AEC, leukocyte count and PELs were significantly different between the different CAD subgroups. Compared to SAP, UAP and non-CAD groups, AEC and PELs in the NSTEMI group were significantly lower, but leukocyte counts were higher, and the most significant differences were found in the ASTEMI group (Table 1). However, there were no significant differences in AEC, leukocyte count and PELs between SAP, UAP and non-CAD groups. Furthermore, compared to non-CAD patients, the mean Gensini score was significantly higher (4.35 ± 3.31 *vs.* 39.39 ± 31.95, $p < 0.001$) in CAD patients. There were also significant differences in the Gensini score between CAD subgroups. The mean Gensini score was significantly higher in the NSTEMI and ASTEMI groups than in the SAP and UAP groups (Table 1).

Most CAD patients were male, elderly, current smokers, and more likely to suffer from diabetes mellitus and hypertension. They also had higher HbA1C, creatinine, uric acid, homocysteine (HCY), and hs-CRP levels, and lower HDL-C and apoA1 levels than non-CAD patients. Moreover, CAD patients received beta blockers ($p < 0.001$), statins ($p < 0.001$) and ACEI/ARB ($p < 0.001$) more frequently.

3.2. Relationship between eosinophil count and CAD

As is shown in Table 2, patients in the highest PELs tertile had significantly higher AECs and lower leukocyte counts than those in the lower tertiles, indicating that reducing ACE and increasing leukocyte count may decrease PELs. Our data also showed a significant relationship between PEL tertiles and the type and severity of CAD; the higher the PELs tertile, the lower the Gensini score ($p < 0.001$, Table 2). There was a significant negative correlation between PELs and Gensini scores ($r = -0.112$, $p < 0.001$). We also observed that there was a higher proportion of non-CAD ($p < 0.001$) and angina pectoris ($p < 0.001$) and a lower proportion of NSTEMI ($p < 0.001$) and ASTEMI ($p < 0.001$) in the highest tertile of PELs.

In addition, PELs also significantly positively correlated with serum creatinine and uric acid ($p < 0.001$) levels, while they significantly negatively correlated with TC, LDL-C, HDL-C, hs-CRP ($p < 0.001$), and apoA1 ($p = 0.020$) levels. Moreover, the higher PELs were significantly associated with male sexes ($p < 0.001$), aged patients ($p < 0.001$), current smokers ($p = 0.001$) and less frequent statin ($p = 0.019$) treatment.

In order to eliminate the impact of residual confounding factors, we performed a multivariable logistic regression analysis (Table 3). After adjusting for baseline differences, low PELs remained strongly associated with severe CAD ($p < 0.001$) and NSTEMI/ASTEMI ($p < 0.001$).

Table 2
Clinical characteristics categorised by tertile of PELs.

	PELs (%)			*p* value
	Tertile 1 (≤1.00, N = 1946)	Tertile 2 (1.01–2.00, N = 1662)	Tertile 3 (2.01+, N = 1679)	
Age (yr)	59.90 ± 10.66	61.19 ± 9.98	62.08 ± 9.74	< 0.001
Male, n (%)	1272(65.4)	1083(65.2)	1230(73.3)	< 0.001
Hypertension, n (%)	1075(55.2)	974(58.6)	947(56.4)	0.123
DM, n (%)	625(32.1)	522(31.4)	537(32.0)	0.893
Current smoker, n (%)	891(45.8)	784(47.2)	873(52.0)	0.001
Family history, n (%)	399(20.5)	310(18.7)	300(17.9)	0.114
Previous PCI, n (%)	423(21.7)	408(24.5)	440(26.2)	0.006
Gensini score	36.04 ± 33.40	30.94 ± 31.75	29.11 ± 29.60	< 0.001
Biochemical parameters				
Total cholesterol, mmol/L	3.89 ± 0.98	3.81 ± 0.95	3.67 ± 0.99	< 0.001
LDL-C, mmol/L	2.29 ± 0.84	2.20 ± 0.81	2.10 ± 0.79	< 0.001
HDL-C, mmol/L	0.99 ± 0.24	0.98 ± 0.25	0.95 ± 0.22	< 0.001
Triglycerides, mmol/L	1.58 ± 1.08	1.59 ± 1.22	1.50 ± 0.97	0.126
HbA1C, %	5.91 ± 1.67	5.85 ± 1.75	5.79 ± 1.56	0.367
Creatinine, μmol/L	63.69 ± 18.96	65.40 ± 35.50	67.36 ± 27.41	< 0.001
Uric acid, μmol/L	306.94 ± 83.66	315.56 ± 79.40	326.45 ± 85.34	< 0.001
HCY, μmol/L	21.19 ± 14.75	20.01 ± 11.61	20.38 ± 13.06	0.448
hs-CRP, mg/L	3.10 ± 3.34	2.55 ± 3.12	2.47 ± 2.93	< 0.001
apoA1, g/L	1.12 ± 0.19	1.12 ± 0.19	1.10 ± 0.18	0.020
Leukocyte, 10^9/L	7.91 ± 2.99	6.63 ± 1.94*	6.46 ± 1.78*#	< 0.001
AEC, 10^9/L	0.04 ± 0.02	0.10 ± 0.03*	0.23 ± 0.13*#	< 0.001
CAD subtypes				
Non-CAD	336 (17.3)	364 (21.9)	380 (22.6)	< 0.001
SAP	90 (4.6)	95 (5.7)	101 (6.0)	0.146
UAP	915 (47.0)	978 (58.8)	1019 (60.7)	< 0.001
NSTEMI	125 (6.4)	65 (3.9)	57 (3.4)	< 0.001
ASTEMI	480 (24.7)	160 (9.6)	122 (7.3)	< 0.001
Concomitant therapy				
Aspirin, n (%)	1895 (97.4)	1611 (96.9)	1616 (96.2)	0.147
Clopidogrel/Ticagrelor, n (%)	1879 (96.6)	1616 (97.2)	1618 (96.4)	0.335
Beta blockers, n (%)	1539 (79.1)	1332 (80.1)	1304 (77.7)	0.210
Statins, n (%)	1892 (97.2)	1618 (97.4)	1609 (95.8)	0.019
ACEI/ARB, n (%)	1539 (79.1)	1350 (81.2)	1323 (78.8)	0.158

DM, diabetes mellitus; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HbA1C, glycated hemoglobin; HCY, homocysteine; hs-CRP, high sensitive C reactive protein; apoA1, apolipoprotein A1; AEC, absolute eosinophil count; PELs, percentages of eosinophils in leukocytes; SAP, stable angina pectoris; UAP, unstable angina pectoris; NSTEMI, non-ST-elevation myocardial infarction; ASTEMI, acute ST-elevation myocardial infarction; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker.
*$p < 0.05$ *vs*. Tertile 1; #$p < 0.05$ *vs*. Tertile 2.

3.3. PELs in discriminating CAD severity

To analyse the contribution of PELs in discriminating CAD severity or NSTEMI/ASTEMI, we performed area under the ROC curve analyses for the predictive model using only PELs or traditional cardiovascular risk factors or a combined model that used PELs and traditional risk factors (Table 4 and Fig. 1). ROC analysis for severe CAD showed that the AUC was only 0.561 (95% CI 0.547–0.574, $p < 0.001$) when the traditional cardiovascular risk factors model was calculated alone, but it improved to 0.658 (95% CI 0.645–0.671, $p < 0.001$) when the combined model was calculated (Fig. 1A). For NSTEMI/ASTEMI, ROC analysis proved that adding PELs data to the traditional risk factor predictive model, the AUC improved from 0.687 (95% CI 0.674–0.700, $p < 0.001$) to 0.756 (95% CI 0.744–0.768, $p < 0.001$) (Fig. 1B). Nonparametric analyses show that the combined model had significantly greater ability in predicting severe CAD ($z = 4.470$, $p < 0.001$) or NSTEMI/ASTEMI ($z = 9.435$, $p < 0.001$) than the model consisting of only traditional risk factors.

4. Discussion

The present study explored the relationship between PELs and CAD and PEL's ability to predict CAD severity or acute coronary arterial thrombotic event. Our results show CAD patients exhibited lower PELs than non-CAD patients. There is a strong association between PELs and the type and severity of CAD, even after adjusting for baseline differences, as seen in the multivariable logistic regression analysis. Moreover, low PELs provided additional predictive value for models that used traditional risk factors to predict severe CAD or acute coronary arterial thrombotic event.

Previous studies have demonstrated that allergic asthma accelerates atherosclerosis plaque formation in mice [2,3], and patients with allergic have a high risk of developing atherosclerosis [6]. As one of the main effector cells of allergic asthma, eosinophils are likely to become novel biomarkers for risk stratification of patients with CAD because of their low cost and easy availability. However, the relationship between eosinophils and CAD remains inconsistent. In the present study, we found there was a significant linear relationship between Gensini score and the PEL tertiles, and low PELs were consistently strongly associated with CAD types and severities. This is consistent with the findings of Jiang et al., [11] but inconsistent with the findings of Verdoia et al. [8,13] Verdoia et al. reported that eosinophil levels are not independently associated with the prevalence and extent of CAD. We considered this inconsistency may be due to severe CAD being defined as left main-three blood vessels in their study, but we defined it as the top Gensini score tertile. Due to the Gensini score system's objectivity in evaluating the coronary arterial stenosis of each segment, our results are accurate and robust.

The level of elevated leukocyte count is directly associated with increased coronary heart disease and ischemic stroke incidence and cardiovascular disease mortality [19]. Therefore, we measured patients' leukocyte count. Compared to the SAP, UAP, and non-CAD groups, AEC

Table 3
Multivariable logistic regression analysis predicts severe CAD and NSTEMI/ASTEMI.

	Severe CAD			NSTEMI/ASTEMI		
	OR	95% C.I.	*p* value	OR	95% C.I.	*p* value
Gender	1.256	0.990–1.592	0.060	1.382	1.021–1.871	0.036
Hypertension	1.155	0.979–1.362	0.088	0.787	0.642–0.964	0.021
Current smoke	1.447	1.185–1.766	0.000	1.104	0.864–1.409	0.429
Family history	1.043	0.858–1.268	0.673	1.905	1.512–2.401	0.000
DM	1.174	0.973–1.417	0.094	0.861	0.680–1.089	0.210
Age	1.022	1.014–1.030	0.000	1.003	0.993–1.013	0.621
Previous PCI	1.232	1.030–1.474	0.022	1.307	1.048–1.629	0.017
Leukocyte	1.090	1.053–1.128	0.000	1.323	1.266–1.383	0.000
PELs	0.897	0.850–0.946	0.000	0.728	0.670–0.791	0.000
Triglycerides	0.941	0.868–1.021	0.145	1.005	0.915–1.103	0.922
LDL-C	1.314	1.185–1.456	0.000	1.440	1.271–1.631	0.000
Creatinine	1.002	0.999–1.004	0.187	0.996	0.991–1.001	0.114
Uric acid	0.999	0.998–1.000	0.289	0.999	0.998–1.000	0.123
hs-CRP	1.089	1.061–1.117	0.000	1.213	1.178–1.249	0.000
HCY	1.005	0.999–1.011	0.110	1.009	1.002–1.016	0.009
HbA1C	1.100	1.044–1.159	0.000	1.082	1.015–1.154	0.016
apoA1	0.297	0.185–0.477	0.000	0.217	0.120–0.394	0.000
aspirin	0.447	0.171–1.168	0.100	0.438	0.129–1.483	0.185
Clopidogrel/Ticagrelor	2.528	0.979–6.530	0.055	5.184	1.411–19.042	0.013
Beta blockers	1.329	1.074–1.645	0.009	1.009	0.776–1.311	0.948
Statins	0.790	0.397–1.572	0.502	0.810	0.328–2.000	0.648
ACEI/ARB	1.583	1.271–1.971	0.000	1.202	0.921–1.569	0.175

TC and HDL-C were not included in the model due to the existence of multicollinearity problems (TC *vs.* LDL-C; HDL-C *vs.* apoA1).
DM, diabetes mellitus; LDL-C, low-density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; HbA1C, glycated hemoglobin; HCY, homocysteine; hs-CRP, high sensitive C reactive protein; apoA1, apolipoprotein A1; PELs, percentages of eosinophils in leukocytes; ACEI, angiotensin-converting-enzyme inhibitor; ARB, angiotensin receptor blocker.

in the myocardial infarction group was significantly lower, but leukocyte counts were higher. There were no significant differences in AEC and leukocyte count between the SAP, UAP, and non-CAD groups. The AEC and leukocyte counts also had a strong negative correlation in different PELs tertiles groups. Considering our study's definition of the PELs, the reduction of PELs maybe caused by a combination of decreased ACE and increased leukocyte counts.

Although our data does not allow us to clarify the direct role of eosinophils in coronary atherosclerosis, the following may explain the underlying relationship between low PELs in peripheral circulation and CAD severity. Firstly, it is well known that ACS is caused by the rupturing of vulnerable atherosclerotic plaque in coronary arteries, which induces thrombus formation and inhibits blood flow to the myocardium, resulting in necrosis [20,21]. Previous studies have shown that eosinophils contribute to intravascular thrombosis by exhibiting a strong endogenous thrombin-generation capacity that relies on enzymatic generation [22]. Eosinophils plays an important role in the initiation, progression, and rupture of thrombus [23], and also in coronary occlusion by promoting thrombus growth [24]. The histologic analysis of tissue samples obtained through thrombus aspiration therapy of ACS patients has shown a large amount of eosinophil infiltration in thrombi [24]. In our study, we observed a strong negative correlation between eosinophil count and CAD severity; the lower the eosinophil count, the more severe CAD. We also found the eosinophil count in the NSTEMI/ASTEMI group were significantly lower compared with the other CAD subgroups. Therefore, we cautiously speculate that the decrease in peripheral-circulating eosinophils may have been induced by extensive thrombus formation; however, the underlying mechanism requires further studiess. Secondly, ECP levels are significantly higher in acute coronary syndrome cases and can independently predict CAD severity [14]. ECP may be released from circulating eosinophils as a response to mediators, which are activated in the inflammatory reaction [25]. In this case, eosinophils cannot be recognized in circulation because of their active participation in the inflammatory process [26]. Thirdly, an increase in cortisol concentration caused by acute stress reactions in STEMI may also result in a decrease in peripheral eosinophil count [27,28]. Finally, eosinophils activated by oxidized LDL could polarize macrophage phenotypes from M2 to M1 through activation of CD36 scavenger receptor [29], and could modulate macrophage function favouring foam cell formation by releasing interferon-α and interferon-β [30]. This may be the possible mechanism for eosinophils involvement in atherosclerosis.

Table 4
ROC curve analyses for the different predictive models.

	AUC	95% CI	*p* value	Sensitivity (%)	Specificity (%)
For severe CAD					
PEL value only	0.561	0.547–0.574	< 0.001	47.44	62.55
Traditional risk factors	0.638	0.625–0.652	< 0.001	52.92	67.37
Combined model	0.658	0.645–0.671	< 0.001	62.36	60.26
For NSTEMI/ASTEMI					
PEL value only	0.687	0.674–0.700	< 0.001	53.42	76.67
Traditional risk factors	0.675	0.662–0.688	< 0.001	65.56	62.97
Combined model	0.756	0.744–0.768	< 0.001	58.58	80.65

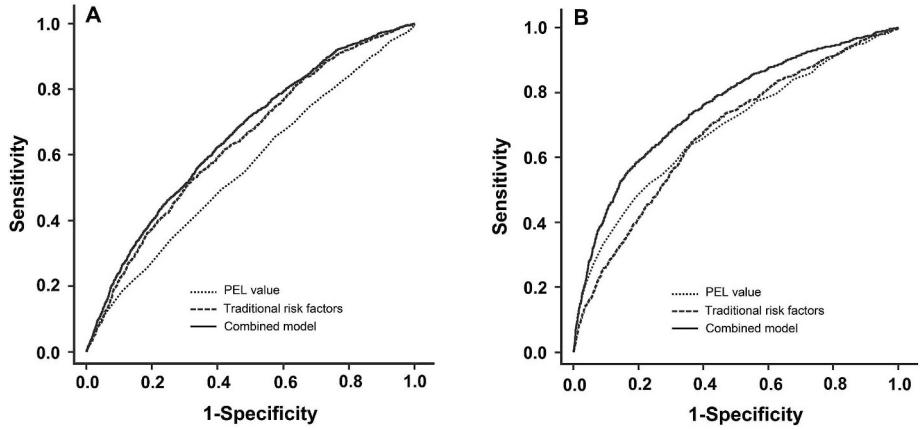

Fig. 1. Area under the ROC curves of low PELs, traditional risk factors, and combined models for predicting severe coronary artery disease (A) or NSTEMI/ASTEMI (B).

This study had a few limitations. Firstly, it is subject to selection bias, as this was a single centre cohort study regarding the Northwest Chinese population and, therefore, may not be applicable to patients from other regions and races. Moreover, our results are only applicable to coronary angiography patients. Further research with a broader study population is required to overcome selection bias. Secondly, this study lacked any long-term follow-ups. Hence, we cannot evaluate the effects of PELs on the long-term prognosis of patients. Finally, regarding the negative correlation between PELs and CAD severity, we also noted some interesting additional results in this study. For example, PELs positively correlated with a few traditional cardiovascular risk factors (serum creatinine, uric acid, male, older, active smoke), while it negatively correlated with a couple of traditional protection factors (HDL-C and apoA1). These results are not consistent with classical views and the potential mechanism on how this occurs requires further research for clarification.

In conclusion, there is likely a strong and consistent negative correlation between PELs and the subtype and severity of CAD, at least in patients who have undergone coronary angiography. This was observed in the multivariable logistic regression analysis when this correlation was seen even after adjusting for baseline differences. Therefore, eosinophils may be a promising biomarker that is cheap, easy to obtain and can accurately and independently predict CAD severity and acute coronary arterial thrombotic event.

Conflicts of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Financial support

This work was supported by the National Natural Science Foundation of China (81500389 to Wang LJ, 81700447 to Gao SS, 91639301 to Yuan ZY); the Natural Science Foundation of Shaanxi Province, China (2018JM7106); and the Clinical Research Award of the First Affiliated Hospital of Xi'an Jiaotong University, China (XJTU1AF-CRF-2018-010 to Gao SS, XJTU1AF-CRF-2016-004 to Yuan ZY).

Author contributions

Zuyi Yuan and Lijun Wang designed the study. Shanshan Gao and Lijun Wang conducted statistical analyses and wrote the manuscript. Jun Wu, Lisha Zhang and Fuxue Deng were involved in the collection and analysis of the data. Juan Zhou and Lijun Wang were involved in the interpretation of results. Yangyang Deng was involved in the collection and analysis of newly added data in the revised manuscript, and did a lot of work for the revision and interpretation of results. All authors reviewed and revised the manuscript.

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