

Allergic Inflammation Is Associated With Coronary Instability and a Worse Clinical Outcome After Acute Myocardial Infarction

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Background—The role of allergic inflammation in acute coronary syndromes (ACS) has not been clearly defined to date. Aim of this study was to assess eosinophil and basophil activation in ACS and the prognostic role of eosinophil cationic protein in ST-segment–elevation myocardial infarction.

Methods and Results—In a cross-sectional study, we prospectively enrolled 51 patients undergoing percutaneous coronary intervention (60.8% patients with ACS and 39.2% with stable angina). Flow cytometry analysis assessed CD66b, CD69, and CD203c median fluorescence intensity expression. In a follow-up study, 181 patients presenting with ST-segment–elevation myocardial infarction, undergoing primary percutaneous coronary intervention, were prospectively enrolled with a follow-up of 24 months. Eosinophil activation (CD66b) was similar in patients with ACS and stable angina (6.61 [4.91–7.72] versus 6.62 [5.27–8.73], $P=0.63$), whereas eosinophil degranulation (CD69) and basophil activation (CD203c) were higher in ACS patients compared with stable angina patients (1.38 [1.16–1.52] versus 1.17 [1–1.31], $P=0.01$); 0.97 [0.89–1.11] versus 0.92 [0.87–0.95], $P=0.03$, respectively). Eosinophil cationic protein serum levels were significantly higher in ST-segment–elevation myocardial infarction patients with major adverse cardiac events as compared with those without (21.1 [10.37–25.65] versus 7.83 [3.37–12.8] $\mu\text{g/L}$, $P=0.01$) and in patients with thrombus score >3 compared with those with thrombus score ≤ 3 (15.0 [9.8–24.7] versus 5.2 [3.5–22.9] $\mu\text{g/L}$, $P=0.006$). Eosinophil cationic protein serum levels predicted major adverse cardiac events during follow-up (odds ratio =1.041, 95% confidence interval 1.012–1.071, $P=0.005$). C-reactive protein serum levels showed a borderline statistical significance (odds ratio =0.904, 95% confidence interval 0.806–1.014, $P=0.085$).

Conclusions—These findings are the first demonstration of in vivo eosinophil degranulation and basophil activation during ACS and of the prognostic role of eosinophil cationic protein in ST-segment–elevation myocardial infarction. (*Circ Cardiovasc Interv.* 2015;8:e002554. DOI: 10.1161/CIRCINTERVENTIONS.115.002554.)

Key Words: acute coronary syndrome ■ allergic cells ■ inflammation

Plaque rupture with activation of inflammatory cells and thrombus formation is the most frequent mechanism causing acute coronary syndromes (ACS). Among inflammatory cells, in the last decades, the scientific community has focused its attention mostly on the role of neutrophils and lymphocytes in the pathogenesis of coronary instability.^{1,2}

However, some studies have suggested that, at least in a subset of patients, eosinophils and basophils also may play a pathogenetic role.^{3,4} These cells, indeed, are rich in small granules, which contain many chemical mediators, released by a process called degranulation following activation of

the cell. So, this process can favor thrombus formation and enhance vasoconstriction at coronary level.^{5,6}

Importantly, eosinophils have been recently involved in thrombus growth and coronary vasospasm.^{7,8} Of note, we reported that higher eosinophil cationic protein (ECP) levels were associated to the atherosclerotic burden and to a worse prognosis in patients undergoing stent implantation.^{9,10} Moreover, in a postmortem study, eosinophil infiltration was found at the site of late drug-eluting stent thrombosis.¹¹

However, to date, the role of eosinophils in coronary instability has not been addressed in detail. Furthermore, the

Received February 3, 2015; accepted July 6, 2015.

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The Data Supplement is available at <http://circinterventions.ahajournals.org/lookup/suppl/doi:10.1161/CIRCINTERVENTIONS.115.002554/-DC1>.

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Circ Cardiovasc Interv is available at <http://circinterventions.ahajournals.org>

DOI: 10.1161/CIRCINTERVENTIONS.115.002554

WHAT IS KNOWN

- Plaque rupture with activation of inflammatory cells and thrombus formation is the most frequent mechanism causing acute coronary syndromes.
- Higher eosinophil cationic protein levels have been associated to the atherosclerotic burden and to a worse prognosis in patients undergoing stent implantation.

WHAT THE STUDY ADDS

- Degranulation of eosinophil cells and activation of basophil cells are involved in the pathogenesis of acute coronary syndrome.
- Serum levels of eosinophil cationic protein predicted major adverse cardiac events at 24 months in a population of patients with ST-segment-elevation myocardial infarction, independently of baseline left ventricular ejection fraction.
- This predictive role of eosinophil cationic protein might be explained by the higher thrombus score associated to raised eosinophil cationic protein levels.

prognostic role of ECP has not been evaluated specifically in patients experiencing ST-segment-elevation myocardial infarction (STEMI), having the largest thrombus burden in the setting of ACS.

Thus, we aimed at assessing the activation of eosinophils and basophils, by flow cytometry, in ACS and in stable angina (SA). Also, we studied, in a STEMI population, the prognostic role of systemic levels of ECP in predicting the occurrence of major adverse cardiac events (MACEs).

Methods

Study 1: Flow Cytometry Study

Study Population

We enrolled 99 patients undergoing coronary angiography for ACS or SA, referred to our Institution between September 2011 and March 2012. ACS included unstable angina, non-STEMI, and STEMI. SA was defined as lack of episodes of coronary instability for at least 6 months before admission. Non-STEMI-ACS patients had at least 2 episodes of angina at rest or one episode lasting 20 minutes during the preceding 48 h, ST-segment shift diagnostic for myocardial ischemia during angina attacks, and abnormal high-sensitivity troponin T levels, according to European guidelines. The diagnosis of STEMI was based on the following criteria: typical chest pain lasting >30 minutes and unresolved by glyceryl trinitrate, ST-segment-elevation ≥ 0.2 mV in at least 2 contiguous leads in the initial ECG, and elevated serum troponin T levels. Exclusion criteria were severe chronic heart failure (New York Heart Association class III–IV; $n=10$ patients), severe valvular disease ($n=5$ patients), systemic inflammatory disease as acute and chronic infections ($n=2$ patients), autoimmune diseases ($n=5$ patients), liver diseases ($n=1$ patient), neoplasia ($n=1$ patient), evidence of immunologic disorders ($n=2$ patients), use of nonsteroidal anti-inflammatory drug and steroids ($n=6$ patients), and recent (<3 months) surgical procedures or trauma ($n=2$ patients). Patients with in-stent restenosis of drug-eluting stents and bare metal stents were excluded. Also patients with stent implantation in the last 12 months before the start of the study were excluded to avoid potential effects of previously implanted stents

on allergic inflammation cells ($n=14$ patients). Patients with a history of allergy were not excluded from the study ($n=4$ patients). In all patients, cardiovascular risk factors were carefully examined, including family history of early coronary artery disease (first degree relative with a history of myocardial infarction <60 years), diabetes mellitus (fasting blood glucose >126 mg/dL or treated diabetes mellitus), dyslipidemia (low-density lipoproteins >130 mg/dL, high-density lipoproteins <45 mg/dL, triglycerides >150 mg/dL, or total cholesterol >200 mg/dL), smoking, and hypertension (systolic blood pressure >140 mmHg and diastolic blood pressure >90 mmHg or treated hypertension). Therapy on admission and angiographic data, including culprit vessel and presence of multivessel disease (≥ 2 diseased vessels with stenosis $\geq 30\%$ by visual estimation), were also recorded.

Left ventricular ejection fraction was calculated at baseline and at discharge using the biplane Simpson's rule, as recommended by the American Society of Echocardiography.

An institutional review committee approved the study, and all the subjects gave informed consent.

Laboratory Assays

Venous blood samples were drawn before coronary angiography. Blood samples were drawn from a brachial vein in all patients and collected in EDTA tubes and centrifuged. Plasma and serum aliquots were stored at -80°C in appropriate cuvettes until assayed. Complete hemochrome, blood glucose, lipid profile, fibrinogen, creatinine, creatine kinase-MB, and troponin T were evaluated using standard methods. C-reactive protein was also measured by an ultrasensitive nephelometric method (DADE-Behring Latex BN-2; lower detection limit, 0.175 mg/L).

All patients gave their consent to the study, and biological measurements were available for all enrolled patients.

Flow Cytometry Analysis

Whole blood was incubated with anti CD16 PE (Beckman Coulter, Brea, CA), CD66b FITC, CD69 PE-Cy7 (BD bioscience, Mountain View, CA), CD45 PE-Cy5 (Beckman Coulter, Brea, CA), and CD203c PE (BioLegend San Diego, CA). Erythrocytes were lysed using Coulter Q-Prep immunology workstation (Beckman Coulter, Brea, CA). At least 100 000 events were acquired. Fluorescence-activated cell sorting (FACS) analysis was conducted with FC 500 (Beckman Coulter, Brea, CA), and the data were analyzed with Kaluza software (Beckman Coulter, Brea, CA).

For eosinophil analysis, a first gate using forward and side scatter characteristics of granulocytes was defined. Then, from these cells, eosinophils were selected as CD16-negative cells. Within this gate, the eosinophil activation was assessed by CD66b and CD69 expression measured as median fluorescence intensity (MFI). Basophil activation was determined measuring CD203c MFI in CD45-positive cells using forward and side scatter characteristics of lymphocytes.

MFI was assessed by dividing the MFI of CD66b, CD69, and CD203c with MFI of the respective isotype controls.

Study 2: Prognostic Study

Study Population

From January 2011 to March 2012, all patients admitted to our hospital with a first episode of STEMI undergoing successful primary percutaneous coronary intervention (PPCI) were screened. Exclusion criteria were symptoms duration >12 hours ($n=6$), age <18 years ($n=0$), history of renal failure (serum creatinine >3 mg/dL; $n=5$), contraindications to contrast agents ($n=1$) or other study medications ($n=0$), paced rhythm ($n=4$), frequent ventricular ectopy ($n=5$), left bundle branch block ($n=6$), preexcitation or other conditions or artifacts interfering with interpretation of ST-segment ($n=2$), rescue PPCI ($n=4$), culprit lesion located in a by-pass graft ($n=5$), stent thrombosis ($n=8$), culprit lesion nonidentified ($n=1$), left main disease ($n=7$), and cardiogenic shock ($n=2$). Overall, 181 patients presenting with STEMI, undergoing PPCI, were enrolled in the study. All patients were treated with aspirin (300 mg) and clopidogrel (600 mg) on admission in the emergency room. All PPCI were performed through a radial or femoral access according to operator preference using a

6 french catheter. A bolus of 5000 UI of heparin was administered. Manual thrombus aspiration and glycoprotein IIb/IIIa inhibitors after diagnostic angiogram at the starting of PPCI (intravenous bolus administration of abciximab, 0.25 mg/kg with a 12-hour infusion after the bolus) were used in all patients. In all patients, cardiovascular risk factors were carefully examined according to the same criteria described before in the flow cytometry study. Time to PPCI, therapy on admission, and angiographic data, such as culprit vessel, thrombus score, and presence of multivessel disease (≥ 2 diseased vessels with stenosis $\geq 30\%$ by visual estimation), were also recorded. An institutional review committee approved the study, and all the subjects gave informed consent.

Clinical Follow-Up and End Points

A clinical follow-up was planned 24 months after discharge, and data about the follow-up were available for all the patients. End points of the study were the composite of cardiac death, recurrent myocardial infarction, and target lesion revascularization, all defined as MACEs. Cardiac death was ascertained by contacting the family doctor or the hospital where the patient died. Recurrent myocardial infarction was defined according to the Third Universal Definition of Myocardial Infarction.¹² Target lesion revascularization was defined as repeat symptom- or ischemia-driven revascularization by either PCI or by coronary artery bypass graft for significant ($>50\%$) in-stent or in-segment (5 mm proximally or distally) restenosis.

Laboratory Assays

Venous blood samples were drawn before coronary angiography and at 1 year after the index procedure from a brachial vein in all patients, then collected in EDTA tubes and centrifuged. Plasma and serum aliquots were stored at -80°C in appropriate cuvettes until assayed. Complete hemochrome, blood glucose, lipid profile, fibrinogen, creatinine, creatine kinase-MB, and troponin T were evaluated using standard methods. C-reactive protein was also measured by an ultrasensitive nephelometric method (DADE-Behring Latex BN-2; lower detection limit, 0.175 mg/L). ECP was measured by enzyme-linked immunosorbent assay (UniCap; Phadia, Uppsala, Sweden) and expressed as $\mu\text{g/L}$; the range of detection was 0.5 to 200 $\mu\text{g/L}$. A cut-off for ECP was defined at 20 $\mu\text{g/L}$.^{13,14}

All patients gave their consent to the study, and biological measurements were available for all enrolled patients.

Statistical Analysis

Continuous variables were expressed as mean $\pm$ standard deviation or median (first to third quartile) if they followed a normal or non-normal distribution, respectively, whereas dichotomous variables were expressed as percentages. Unpaired *t* test or Mann-Whitney U-test was used for the comparison of continuous variables; categorical variables were compared between groups using the χ^2 test or Fisher exact test, as appropriate. Correlations between continuous variables were analyzed using Pearson or Spearman test, as appropriate. Independent predictors of MACEs at follow-up were identified by a multivariable Cox regression analysis model, including the variables showing a *P* value <0.10 at univariable Cox regression analysis. Hazard ratio (HR), odds ratio, and 95% confidence interval (CI) have been reported. Survival curves using the Kaplan-Meier methods were produced for ECP according to the cut-off value (20 $\mu\text{g/L}$) and compared by the logrank test. A 2-tailed *P* value <0.05 was the level of statistical significance. All analyses were performed using the SPSS 20.0 software (Florence, Italy).

Results

Study 1

Among the overall population of 51 patients, 31 (60.8%) presented ACS and 20 (39.2%) SA. The 2 study groups presented similar clinical characteristics and laboratory and angiographic data as reported in Table 1. C-reactive protein, creatin kinase MB, and troponin T serum levels were all significantly higher

in ACS patients compared with patients with SA. By flow cytometry, patients with ACS had similar eosinophil activation (CD66b; 6.61 [4.91–7.72] versus 6.62 [5.27–8.73] MFI, *P*=0.63), but higher eosinophil degranulation (CD69) and basophil activation (CD203c) as compared with SA patients, respectively (1.38 [1.16–1.52] versus 1.17 [1.00–1.31] MFI, *P*=0.01 and 0.97 [0.89–1.11] versus 0.92 [0.87–0.95] MFI, *P*=0.03; Figure 1). Conversely, eosinophil count was similar between the 2 groups of ACS and SA patients (Table 1), and no correlation was found between eosinophil count and subpopulations of CD66b and CD69 (*P*=ns).

Study 2

Clinical characteristics, therapy on admission, laboratory and angiographic data of the overall STEMI population, and univariable COX regression are reported in Table 2. At 24 months clinical follow-up, among the overall population, 60 (33.1%) patients experienced MACEs: 19 cardiac deaths, 19 recurrent myocardial infarctions, and 22 target lesion revascularizations.

Patients who developed MACEs had a lower basal echocardiographic ejection fraction than patients who did not (HR=0.97; 95% CI, 0.94–0.99; *P*=0.032). C-reactive protein serum levels tended to be higher in patients with MACEs than in those without (HR=0.93; 95% CI, 0.9–1.01; *P*=0.077). Notably, ECP serum levels were significantly higher in patients with MACEs as compared with those without (HR=1.59; 95% CI, 0.95–2.67; *P*=0.01); in particular, patients who presented MACEs had more frequently ECP levels $>20 \mu\text{g/L}$ as compared with those without MACEs (HR=2.72; 95% CI, 1.63–4.51; *P*<0.001). Correlates of ECP levels are shown in Tables I and II in the Data Supplement.

Of note, ECP levels were significantly higher in patients with thrombus score 4 or 5 as compared with those with thrombus score ≤ 3 (15 [9.88–24.7] versus 5 [3.46–22.96] $\mu\text{g/L}$, *P*=0.006). Conversely, no difference in eosinophil count was found according to the occurrence of MACEs. Moreover, no correlation was found between eosinophil count and ECP levels (*P*=ns).

At a multivariable analysis, lower values of baseline echocardiographic ejection fraction and higher levels of ECP serum levels were significantly associated with a higher rate of MACEs during follow-up (odds ratio =0.957; 95% CI, 0.922–0.994; *P*=0.023 and odds ratio =1.041; 95% CI, 1.012–1.071; *P*=0.005, respectively), whereas higher C-reactive protein serum levels were of borderline statistical significance (odds ratio =0.904; 95% CI, 0.806–1.014; *P*=0.085).

A Kaplan-Meier analysis on estimated free survival curve of MACEs, during 24 months clinical follow-up, showed worse MACEs free survival curve of STEMI patients with ECP serum levels $>20 \mu\text{g/L}$ as compared with patients with ECP serum levels $<20 \mu\text{g/L}$ (*P*<0.001; Figure 2). Similarly, a Kaplan-Meier analysis with median ECP values (12.8 $\mu\text{g/L}$) as cut-off showed a trend for a worse MACEs free survival curve of STEMI patients with ECP values above the median as compared with patients with ECP values below it (Figure in the Data Supplement).

Discussion

In this study, we demonstrate for the first time that degranulation of eosinophil cells and activation of basophil cells are

Table 1. Baseline Clinical Characteristics, Therapy on Admission, Routine Laboratory Data, and Angiographic Data, According to the 2 Study Groups

Variables	ACS, 31 (61%)	Stable Angina, 20 (39%)	P Value
Clinical characteristics			
Age, y*	66.4±14.3	69.3±12.1	0.45
Hypertension, n (%)†	26 (84)	19 (95)	0.23
Current smoker, n (%)†	17 (55)	8 (40)	0.23
Dyslipidemia, n (%)†	20 (65)	14 (70)	0.46
Diabetes mellitus, n (%)†	5 (16)	5 (25)	0.33
Family history of CAD, n (%)†	7 (23)	5 (25)	0.55
Therapy on admission			
Beta-blockers, n (%)†	20 (65)	9 (45)	0.14
Ace-inhibitors, n (%)†	17 (55)	12 (60)	0.47
Statins, n (%)†	20 (64)	16 (80)	0.19
Aspirin, n (%)†	28 (90)	20 (100)	0.22
Routine laboratory data			
Hemoglobin, g/dL*	14.32±1.51	12.15±7.36	0.11
White blood cells, mm ⁻³ ‡	8890 (7902–10 367)	6550 (4937–8850)	0.19
Neutrophils, %‡	68.10 (59.30–75.10)	58.05 (47.00–66.57)	0.21
Lymphocytes, %‡	24.10 (17.70–31.00)	27.65 (23.82–39.55)	0.30
Monocytes, %‡	6.10 (4.90–7.70)	6.10 (5.37–8.32)	0.39
Eosinophils, %‡	1.20 (0.5–2.50)	1.90 (1.20–2.70)	0.36
Basophil, %‡	0.30 (0.30–0.50)	0.50 (0.30–0.80)	0.15
Platelets, 10 ³ /mm ³ ‡	209 (170–243)	222 (175–237)	0.58
Blood glucose, mg/dL‡	98.5 (87.75–130.00)	95 (87.25–103.75)	0.46
Total cholesterol, mg/dL‡	169 (146–190)	156 (134–181)	0.99
LDL, mg/dL‡	109 (84–130)	96 (66–118)	0.39
HDL, mg/dL‡	40.5 (36.7–45.5)	40.5 (38.2–42.0)	0.39
Triglycerides, mg/dL‡	118 (78–147)	125 (106–168)	0.52
Fibrinogen, mg/dL‡	316 (294–420)	309 (276–384)	0.35
Creatinine, mg/dL*	0.91±0.25	1.02±0.21	0.12
C-reactive protein, mg/L‡	6.45 (3.30–16.35)	2.50 (2.50–3.30)	0.003
Creatine-kinase MB, ng/mL‡	12.1 (2.4–10.8)	2.8 (1.9–3.6)	0.012
Troponin T, ng/mL‡	0.30 (0.10–1.60)	0.011 (0.007–0.02)	0.014
Angiographic data			
Culprit vessel, n (%)†			0.36
Left anterior descending artery	16 (52)	8 (40)	
Circumflex artery	7 (23)	3 (15)	
Right coronary artery	8 (26)	9 (45)	
Multivessel disease†	17 (55)	7 (35)	0.14

Continuous variables are expressed as median (first to third quartile) or mean±standard deviation. ACS indicates acute coronary syndromes; CAD, coronary artery disease; HDL, high-density lipoproteins; LDL, low-density lipoproteins; and multivessel disease, at least 2-vessel disease.

*Unpaired *t* test.

†Chi-squared test.

‡Mann-Whitney test.

involved in the pathogenesis of ACS. In particular, using flow cytometry analysis, we found a greater expression of CD69 and CD203, markers of eosinophil degranulation and of basophil activation, respectively, in patients with ACS compared with in patients with SA, whereas CD66 expression, marker of eosinophil activation, remained similar between the 2 populations of coronary artery disease.

Inflammatory cells, including lymphocytes, macrophages, neutrophils, and eosinophils, are known to play an important role in arterial thrombogenesis.^{1,2}

In particular, eosinophil granule proteins may promote thrombus formation and growth.^{7,15} Of note, CD66b glycoprotein plays an important role in regulating adhesion and activation of eosinophils.¹⁶ Furthermore, CD66b activation induces production and

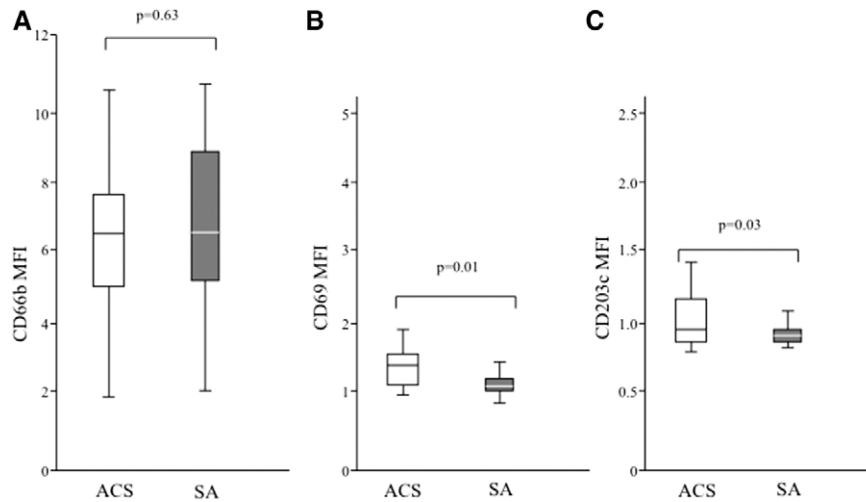


Figure 1. Comparisons of eosinophils activation (CD66b MFI; **A**), eosinophils degranulation (CD69 MFI; **B**), and basophils activation (CD203c MFI; **C**) between acute coronary syndromes (ACS) patients and stable angina (SA) patients. Data are presented as box plots with median and first to third quartiles. ACS indicates acute coronary syndromes; CD203c, marker of basophil activation; CD66b, marker of eosinophil activation; CD69, marker of eosinophil degranulation; MFI, median fluorescence intensity; and SA, stable angina.

release of proinflammatory mediators by human eosinophils.^{16,17} Although CD66b is a versatile positive regulator of eosinophil effector functions, CD69 is not constitutively expressed in blood eosinophils. In fact, previous studies showed that CD69 expression on eosinophils is induced by some eosinophil-active cytokines (granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5). It is also a marker of degranulation in bronchoalveolar liquid of asthmatic patients.¹⁸

As eosinophil activation precedes degranulation, we have probably explored a time window in which eosinophils had been already activated, whereas they are still in the active phase of degranulation. Indeed, most of our patients were assessed hours after chest pain onset.

It is worth noting that CD69 might be a coreceptor in triggering platelet aggregation and degranulation via phospholipase A₂ activation and subsequent release of Thromboxane A₂.^{18,19} This linking with platelet activation supports the notion that CD69 might have a role in plaque destabilization and thrombus formation during ACS.

CD69 is involved in the activation of different leukocyte subsets and play a complex role in immune response. However, although the engagement of this receptor triggers the activation of several signaling pathways, it seems not to be evidence of a molecular shedding by the T-cell surface or by platelets. We cannot exclude the existence of a small fraction of platelets–eosinophils aggregates in our sample.

However, this modest percentage of adherent platelets (generally is around 10% to 15% in nonasthmatic subjects) is expected to not significantly affect the CD69 MFI on eosinophils.

In addition, eosinophils can induce a proinflammatory state in endothelial cells.²⁰ Finally, eosinophils and basophils are source of interleukin-17 recently involved, with controversial data, in coronary instability.^{21,22}

Basophil cells, another important player in allergic-inflammatory responses, have been assessed in our study using CD203c, a specific marker of IgE-dependent basophil activation, rapidly up-regulated after allergen challenge.²³

So, we can speculate that its increase during ACS may be an expression of an early-onset allergic reaction. Indeed, CD203c is a marker of basophil activation and is not necessarily associated with the IgE pathways.²⁴ Of course, the overexpression of CD203c observed in ACS patients does not eliminate the role of IgE in the pathogenesis of the disease.

However, the lack of patients with allergic and inflammatory disease in our study, in whom higher levels of IgE are expected, indicates a minor role of this immunoglobulin in ACS.

Further and deeper studies are needed to determine the implication of IgE and the allergic disorders in ACS. Allergic inflammation, in fact, may play a role in plaque destabilization and rupture⁶ and in ACS associated with hypersensitivity or anaphylactic reactions, in particular, in case of stent implantation.²⁵

Eosinophil activation may be assessed by measuring serum levels of ECP. ECP has been found to be associated with coronary atherosclerotic burden and with a poor clinical outcome after implantation of both bare metal and drug-eluting stents.^{9,10,26}

In this study, we measured ECP in a population of STEMI patients at the time of PPCI. We found that serum levels of ECP predicted MACEs at 24 months, independently of baseline ejection fraction. This predictive role might be, at least partially, explained by the higher thrombus score associated to raised ECP levels.

The relationship between high serum levels of ECP and high thrombus score could be accounted for by the results of previous histopathologic studies in ACS reporting a greater eosinophil infiltration in larger red thrombi. It suggests a possible important role for eosinophils in thrombus growth.⁷

The role of ECP in thrombus growth could be mediated by thrombomodulin inhibition, as in hypereosinophilic syndrome or allergic diseases.²⁷ However, the evidence of an increased expression of the activation marker CD69 on the surface of eosinophils suggests a clear implication of these cells in ECP production.

Table 2. Study Population and Univariable COX Regression

Variables	Overall STEMI Population, N=181 Patients	HR (95% CI)
Clinical characteristics		
Age, y	63.5±12.8	0.99 (0.97–1.01)
Male sex, n (%)	155 (86)	0.62 (0.32–1.16)
Hypertension, n (%)	101 (56)	1.03 (0.61–1.71)
Current smoker, n (%)	98 (54)	1.24 (0.74–2.06)
Dyslipidemia, n (%)	84 (46)	1.36 (0.81–2.25)
Diabetes mellitus, n (%)	43 (24)	1.71 (0.99–2.92)
Family history of CAD, n (%)	65 (36)	1.03 (0.60–1.73)
Ischemic time, min	150 (120–360)	0.99 (0.99–1.00)
LVEF, %	47 (39–55)	0.97 (0.94–0.99)*
Therapy on admission		
Beta-blockers, n (%)	145 (80)	0.87 (0.5–1.6)
Ace-inhibitors, n (%)	121 (67)	1.09 (0.64–1.82)
Statins, n (%)	141 (78)	0.98 (0.59–1.63)
Aspirin, n (%)	70 (39)	1.40 (0.84–2.34)
Hemoglobin, g/dL	13.50±2.10	0.94 (0.84–1.05)
White blood cells, mm ⁻³	11 (10–14)	0.95 (0.88–1.04)
Neutrophils, %	76.20 (59.90–86.60)	1.01 (0.99–1.04)
Lymphocytes, %	17.10 (9.70–29.40)	0.99 (0.96–1.02)
Monocytes, %	4.80 (3.40–6.25)	0.92 (0.79–1.07)
Eosinophils, %	0.81 (0.55–1.07)	0.87 (0.69–1.09)
Basophils, %	0.75 (0.40–1.50)	1.00 (0.67–1.496)
Platelets, 10 ³ /mm ³	238 (208–277)	1.00 (0.96–1.00)
Blood glucose, mg/dL	138.10 (117.00–174.40)	0.99 (0.99–1.00)
Total cholesterol, mg/dL	178 (153–199)	1.00 (0.99–1.01)
LDL, mg/dL	107 (93–134)	1.00 (0.99–1.01)
HDL, mg/dL	42 (38–47)	0.99 (0.95–1.03)
Triglycerides, mg/dL	117 (84–149)	1.002 (0.999–1.005)
Fibrinogen, mg/dL	310 (268–373)	1.001 (0.997–1.004)
Creatinine, mg/dL	1.25±0.78	0.70 (0.31–1.6)
C-reactive protein, mg/L	1.6 0 (0.70–3.30)	0.93 (0.9–1.01)
Creatine-kinase MB, ng/mL	124.5 (54.6–377.0)	1.00 (0.1–1.00)
Troponin T, ng/mL	3.45 (1.45–9.17)	1.02 (0.99–1.06)
ECP serum levels, µg/L	10.70 (4.39–21.30)	1.59 (0.95–2.67)†
ECP levels >20 µg/L, n (%)	57 (31)	2.72 (1.63–4.51)‡
Angiographic data		
Culprit vessel		0.92 (0.69–1.20)
Left anterior descending artery	91 (50)	
Circumflex artery	21 (12)	
Right coronary artery	69 (38)	
Thrombus score >3	121 (67)	1.26 (0.95–1.67)
Multivessel disease	98 (54)	0.87 (0.52–1.45)

Continuous variables are expressed as median (first to third quartile) or mean±standard deviation. CAD indicates coronary artery disease; CI, confidence interval; ECP, eosinophil cationic protein; HDL, high-density lipoproteins; HR, hazard ratio; LDL, low-density lipoproteins; LVEF, left ventricular ejection fraction; and multivessel disease, at least 2-vessel disease.

* $P=0.032$.

† $P=0.01$.

‡ $P<0.001$.

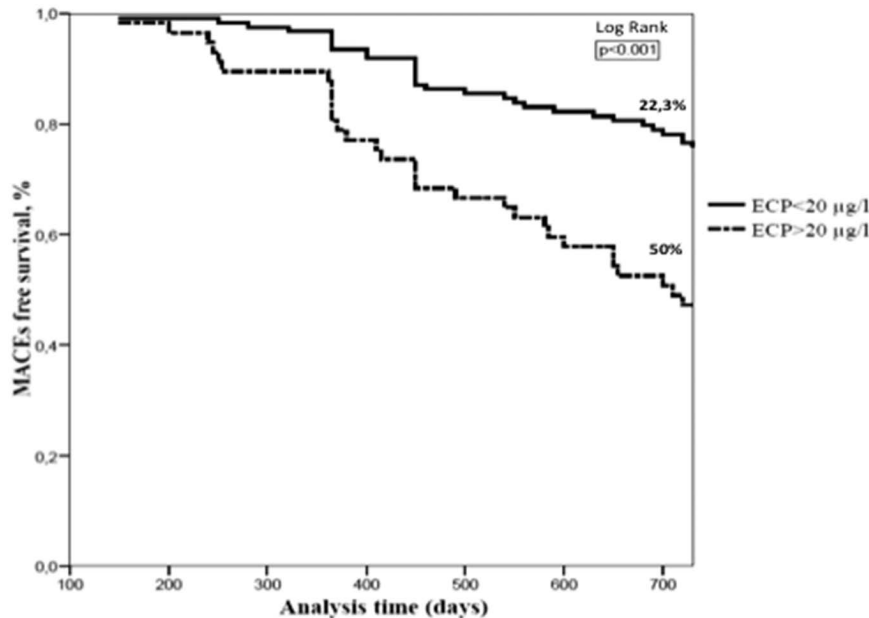


Figure 2. Major adverse cardiac events (MACEs) free survival Kaplan–Meier curves according to eosinophil cationic protein (ECP) levels above or below the cut-off value (20 µg/L). Patients having ECP levels >20 µg/L had a worse MACEs free survival curve when compared with those having ECP levels <20 µg/L ($P < 0.001$).

Indeed, increased CD69 expression is associated with increased release of ECP in activated eosinophils.²⁸ Of course, we cannot exclude the role of platelets activation in the assessment of ECP plasma levels. Larger studies are needed to determine the role of ECP production by platelets in ACS.

Therefore, our findings confirmed the independent role of ECP in predicting long-term MACEs as previously reported in other populations after bare metal and drug-eluting stent implantation.^{10,26}

Our study is focused on an unselected coronary artery disease population and not focused on patients with previous history of allergic disease. Accordingly, median value of ECP (12.8 µg/L) in our population was lower than the upper normal limit (20 µg/L) recommended by allergologist. Nevertheless, by using the median as cut off, a trend for higher MACEs rate was observed in those with ECP levels above the median, whereas a significant difference in MACEs rate was shown when using the allergologist-recommended cut-off.

This finding suggests that patients with higher eosinophil activation, as assessed by ECP, may represent a high-risk population.

The predictive value of ECP might be mainly related to in-stent restenosis as observed in previous studies in other populations. Indeed, target lesion revascularization in this study was a major component of MACEs.¹⁰

Moreover, the lack of correlation between eosinophil count and CD66 and CD69 or ECP levels suggests that eosinophil activation more than eosinophil number should be taken into account for risk stratification of patients presenting with an ACS.

Further studies should focus on patients with history of allergic disease. An important limitation of our study is represented by the sample size of the study.

In particular, considering the modest number of PCI performed (51 patients), as well as MACE (60 patients), too

many statistical tests were performed at nominal 5% significance level, thus inflating a type I statistical error.

Moreover, the lack of assessment of all components of an allergic reaction, including assessment of Th2 T cells and related cytokines, and of IgE antibody levels have to be also taken in account.

Our results, taken together, are the first to suggest that an allergic reaction might play a role in coronary instability, at least in a subset of patients. Thus, our results suggest that therapies targeting eosinophils might be tested in ACS patients exhibiting raised ECP levels.

Few drugs have been tried hitherto, in addition to corticosteroid therapy, in eosinophilic disorders. Mepolizumab, an anti-interleukin-5 antibody, has recently been demonstrated to decrease blood eosinophil levels.²⁹ Of note, statins, such as fluvastatin and lovastatin, have been found to inhibit granulocyte-macrophage colony-stimulating factor-stimulated eosinophil adhesion to intercellular adhesion molecule 1, suggesting an inflammatory role of statins in asthma.³⁰

Disclosures

None.

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