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Pre-intervention eosinophil cationic protein serum levels predict clinical outcomes following implantation of drug-eluting stents

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Aims Eosinophils have been identified in post-mortem studies as important players of both restenosis and thrombosis after drug-eluting stent (DES) implantation. We aimed at assessing the association between baseline levels of eosinophil cationic protein (ECP), a marker of eosinophil activation, and recurrence of clinical events in a consecutive series of patients who underwent DES implantation.

Methods and results Two hundred patients (age 63 ± 10.4, males 75%) undergoing implantation of first-generation DES (Taxus or Cypher stents) were enrolled. We measured serum levels of ECP and total IgE by enzyme-linked immunosorbent assay and of C-reactive protein by high-sensitivity nephelometry prior to percutaneous coronary intervention. A clinical follow-up was planned 18 months after discharge. Major adverse cardiac events (MACEs), such as cardiac death, recurrent myocardial infarction, or clinically driven target lesion revascularization, were the endpoint of the study. Twenty-two patients (11%) had MACEs and showed higher serum levels of ECP compared with those without MACEs [30.5 (14.4–50) vs. 12.2 (4.4–31) μg/L, $P = 0.004$]. At simple Cox regression analysis, serum levels of ECP were a significant predictor of MACEs (hazard ratio 1.016, 95% confidence interval 1.003–1.03, $P = 0.018$).

Conclusion This study shows for the first time an association between baseline ECP levels and the occurrence of MACEs in patients undergoing implantation of DES. Further studies are warranted to establish whether in this setting ECP is a risk marker or plays a contributory pathogenetic role.

Keywords Drug-eluting stent • Eosinophils • Eosinophil cationic protein • Prognosis

Introduction

In the last year, the initial enthusiasm for drug-eluting stent (DES) generated by the lower restenosis rate when compared with bare metal stent (BMS) has partially been replaced by growing concern for the apparently higher risk of death.[1,2] Lack of re-endothelialization and antiplatelet therapy discontinuation have emerged as predisposing factors for subacute and very late stent thrombosis with DES.[3,4] Drug discontinuation, however, explains part of the phenomenon only, thus suggesting that other mechanisms may play an important role.

Inflammation is known to play a key role in the pathogenesis of restenosis,[5] but, while the inflammatory stimulus following BMS implantation is represented by metallic struts only, inflammation following DES implantation is also triggered by the polymer.[6] Interestingly, eosinophils are observed among inflammatory cells infiltrating DES at a higher concentration when compared with BMS.[7,8] These findings suggest that allergy-mediated inflammation may be involved in DES restenosis and thrombosis.

In this prospective study, based on a consecutive series of patients undergoing DES implantation, we aimed at assessing whether baseline levels of eosinophil cationic protein (ECP), a

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sensible marker of allergic inflammation, predict the risk of future major adverse cardiac events (MACEs) after implantation of first-generation DES.

Methods

Patient population and study protocol

Two hundred consecutive patients were included in this study. Those eligible included patients presenting from September to November 2005 with symptomatic stable or unstable ischaemic heart disease (IHD) who underwent successful implantation of a sirolimus (Cypher; Cordis, Johnson & Johnson, Miami Lakes, FL, USA) or paclitaxel-eluting stents (Taxus; Boston Scientific, Boston, MA, USA). Patients were enrolled in the catheterization laboratory just after the operator decision to implant a DES. Overall, 270 patients were initially screened for the study. Exclusion criteria were: acute ST-elevation myocardial infarction (MI; $<$24 h, $n = 30$ patients), severe chronic heart failure (NYHA class III–IV; $n = 10$ patients), severe valvular disease ($n = 5$ patients), systemic inflammatory diseases as acute and chronic infections ($n = 4$ patients), autoimmune diseases ($n = 1$ patient), liver diseases ($n = 1$ patient), neoplasia ($n = 1$ patient), evidence of immunologic disorders ($n = 1$ patient), use of anti-inflammatory or immunosuppressive drugs ($n = 4$ patients), and recent ($<$3 months) surgical procedures or trauma ($n = 3$ patients). Patients with in-stent restenosis of DES and BMS were excluded as well as patients with stent implantation in the last 12 months before the start of the study in order to avoid potential effects of previously implanted stents on ECP levels ($n = 10$ patients). Patients with a history of allergy were not excluded from the study ($n = 11$ patients). No patients refused to consent to the study, and biological measurements were available for all enrolled patients.

In all patients, cardiovascular risk factors were carefully examined. History of IHD was defined as any previous diagnosis of stable or unstable coronary syndromes. All patients received the same DES if more than one lesion per patient was treated. The choice between sirolimus- or paclitaxel-eluting stent was left at operator discretion. All patients received aspirin and clopidogrel (600 mg) at least 2 h before the procedure. After percutaneous coronary intervention (PCI), aspirin was prescribed lifelong and clopidogrel for 9 months.

A clinical follow-up was planned 18 months after discharge, and data about the follow-up were available for all the patients. The endpoint of the study was the composite of cardiac death, MI, and clinically driven target lesion revascularization (TLR). Cardiac death was ascertained by contacting the family doctor or the hospital where the patient died. Myocardial infarction was diagnosed by a more than three-fold elevation of CK-MB levels above upper normal limit associated with typical chest pain. Target lesion revascularization was carried out in the presence of a diameter stenosis $>$50% within 5 mm proximal or distal to the previously implanted stent.

All patients gave their informed consent, and the study was approved by the Ethics Committee of the University.

Coronary angiographic evaluation

An expert angiographer (F.B.), unaware of the laboratory values, evaluated angiographic images both qualitatively and quantitatively. Lesion morphology was assessed by using the modified American College of Cardiology/American Heart Association grading system (type A, B1, B2, and C), whereas coronary artery disease severity by counting the number of coronary artery branches showing at least one critical stenosis ($>$70% reduction in lumen diameter). Digital angiograms were quantitatively analysed offline with the use of an automated edge-detection system (CMS; Medis Medical Imaging Systems, The Netherlands). All measurements were performed on images obtained after intracoronary nitrate administration. The following angiographic parameters were obtained: reference vessel diameter (RVD), minimal lumen diameter (MLD), and diameter stenosis (DS) per cent which were evaluated both at baseline and at the end of the procedure, lesion length, and total stent length. The procedure was considered successful if residual stenosis was $<$30% with TIMI flow grade 3. Four patients were excluded due to failure of the procedure (unsuccessful wire crossing of a chronic total occlusion).

Blood samples and laboratory assay

Blood samples were obtained just prior to PCI. Each venous blood sample was centrifuged in appropriate tubes and stored at -80°C. C-reactive protein (CRP) was measured by an ultrasensitive nephelometric method (DADE-Behring Latex BN-2), with a lower detection limit of 0.2 mg/L. Eosinophil cationic protein and total IgE were measured by enzyme-linked immunosorbent assay (UniCap; Phadia, Uppsala, Sweden) and expressed as μg/L and KU/L, respectively. For ECP serum levels, range of detection was 0.5–200 μg/L and interassay coefficient of variation was 4%;[9,10] for total IgE serum levels, range of detection was 2–5000 KU/L and interassay coefficient of variation was 5.3%.[11]

Statistical analysis

Normal distribution was assessed by the Kolmogorov–Smirnov test. As CRP, ECP, and IgE levels did not follow a normal distribution, they were expressed as median and interquartile range, whereas other continuous variables were expressed as means $\pm$ standard deviation; categorical variables were expressed as proportions. Continuous variables were compared by Student's *t*-test or Mann–Whitney *U* test as appropriate, whereas categorical variables by Fisher's exact test. Correlations between continuous variables were done by the Spearman rank correlation test.

In this study, there is only right censoring of the data, i.e. major adverse cardiac events did not occur in the remaining patients before the end of follow-up and the use of Cox proportional hazard ratio (HR) model is allowed with this type of data. Survival duration was measured from the date of discharge to the occurrence of a MACE event or to the date of last known follow-up evaluation. Further, no patient experienced repeated events at different times of follow-up. For this reason, we did not consider the estimation of the frailty in the patient risk. Thus, as primary analysis we performed a simple Cox regression analysis using all variables on their original continuous scale in order to estimate the unadjusted HRs of all variables. We also calculated the 95% confidence interval (CI) of the coefficient of the Cox regression with bootstrap estimation using the bias-corrected and accelerated method, after 20 000 replications.

To account for intra-patient correlations due to the presence of patients with multiple lesions, we considered the lesion as the individual entity and the patient as a cluster. We used the 'cluster' option available in STATA, i.e. generalization of the Huber-White sandwich estimate of variance[12,13] in which the meat of the sandwich is substituted with a matrix formed by taking the outer product of the cluster-level scores, where within each cluster the cluster-level score is obtained by summing the observation-level scores.[14–16]

As the number of MACEs was 22 in our patient population and the number of variables for inclusion in a multiple Cox regression analysis should be $22/10 = 2$, we refrained from performing a multiple Cox regression analysis due to the high risk of overfitting with any building

model.[17] The validity of the proportional-hazards assumption was tested by adding a time-dependent interaction variable for each of the predictors. The assumption of linearity for continuous variables was confirmed by the use of restrictive cubic spline function.[18]

As secondary analysis, for the continuous variables ECP and IgE levels, we calculated a cut-off value in order to obtain a dichotomous variable associated with MACEs, using the receiver operating characteristic (ROC) curve analysis. For the censoring of the data concerning the ROC analysis of the outcome, we considered the method described by Song and Zhou.[19] The optimal cut-off was chosen on the basis of the maximum value of the sum of sensitivity and specificity.[20] In order to validate our results, we used the bootstrapping procedure for illustrating the uncertainty that surrounds the resulting estimate of the cut-off (debiased 95% CI), using the methods developed by Efron and Tibshirani.[21]

Survival curves using the Kaplan–Meier methods were produced for ECP according to the cut-off value derived from the ROC analysis and compared by the logrank test.

Our study is the first ever examining the association between ECP serum levels and the outcome after DES implantation, thus making it impossible to utilize previous studies for the calculation of sample sizes. Thus, we decided to include 200 patients considering the expected MACE rate (about 10%) previously observed in a similar population. Based on this assumption, we thought that 20 patients experiencing MACEs would have been sufficient in order to demonstrate clinically relevant differences of ECP serum levels between patients with or without MACEs. All analyses were performed using STATA 9.0 (Stata Corporation, College Station, TX, USA) and S-plus.

Results

Main features and clinical outcomes of the study population

Population characteristics are summarized in *Table 1*. We included 200 patients with 226 lesions. Our population reflects a real-world scenario with a high prevalence of acute coronary syndrome (ACS; 51%) and of complex B2/C-type lesions (55%) or multivessel disease (61%). Furthermore, the mean number of stents per patient and the mean stent length reflects our current practice of complete lesion coverage with DES (*Table 1*). Overall, 105 patients received paclitaxel-eluting stents in 118 lesions and 95 patients received sirolimus-eluting stents in 108 lesions.

At follow-up, 22 patients experienced a MACE and two patients died because of neoplasia. Death was of cardiac origin in four patients (2%) (sudden death in one patient, MI in two patients, and acute heart failure with ECG ischaemic changes in one patient; three deaths occurred in patients who received paclitaxel-eluting stent and one death in a patient receiving sirolimus-eluting stent). One patient experienced stent thrombosis of a paclitaxel-eluting stent causing non-fatal ST-elevation MI (0.5%). Seventeen patients (8.5%) experienced clinically driven TLR (12 patients received paclitaxel-eluting stent, whereas five patients received sirolimus-eluting stent). Importantly, 60% of MACEs occurred more than 180 days after DES implantation, and 27% of MACEs occurred after 1 year. No patient discontinued antiplatelet therapy before the prescribed period.

History of allergy was present in 11 patients (seven patients had seasonal respiratory symptoms, three had allergy to antibiotics, and one had alimentary allergy) but none of these patients developed any MACEs at follow-up. Eosinophil cationic protein levels in patients with a history of allergy were similar compared with those in patients without a history of allergy who did not experience MACEs at follow-up (data not shown).

Table 1 Baseline features in the overall patient population

Characteristics	Data
Age (years)	62.6 ± 10.4
Males, *n* (%)	150 (74.6)
Smoking, *n* (%)	58 (28.9)
Diabetes, *n* (%)	60 (29.9)
Hypercholesterolemia, *n* (%)	123 (61.2)
Hypertension, *n* (%)	134 (66.7)
Family history, *n* (%)	80 (39.8)
Acute coronary syndromes, *n* (%)	102 (50.7)
STEMI <3 months, *n* (%)	19 (9.5)
Previous CABG, *n* (%)	21 (10.4)
Previous PCI, *n* (%)	51 (25.4)
Previous IHD, *n* (%)	129 (64.2)
Ejection fraction	55.4 ± 9.6
Multivessel disease, *n* (%)	122 (61)
Stent number (per patient)	1.44 ± 0.7
Stent length (mm)	24 (18–40)
B2/C, *n* (%)	124 (55)
Reference vessel diameter	2.7 ± 0.5
ECP serum levels (μg/L)	14.8 (4.7–33.8)
ECP levels >11 μg/L, *n* (%)	111 (55.5)
Total IgE serum levels (KU/L)	34 (18.4–77)
CRP serum levels (mg/L)	3 (1.1–10)
Abnormal baseline Troponin T, *n* (%)	58 (29)
White cell blood count	7.8 ± 2.26
Eosinophil count	0.14 (0.08–0.22)
Neutrophil count	4.9 ± 1.9
Lymphocyte count	2.1 ± 0.7
Monocyte count	0.5 ± 0.26

STEMI, ST-elevation myocardial infarction; IHD, ischaemic heart disease; ECP, eosinophil cationic protein; CRP, C-reactive protein.

Predictors of major adverse cardiac events and determinants of eosinophil cationic protein levels

Several factors associated with the risk of MACEs were identified (*Tables 2* and *3*). Among clinical variables, a previous history of IHD, diabetes, and a lower ejection fraction tended to be more frequent in patients with MACEs when compared with those without MACEs ($P = 0.11$, 0.12, and 0.07, respectively). Among laboratory data, patients with MACEs had higher serum levels of ECP [30.5 (14.4–50) vs. 12.2 (4.4–31) μg/L, $P = 0.004$, *Figure 1*] and a trend for higher serum levels of IgE [50 (27.6–208) vs. 33 (17.4–65) KU/L, $P = 0.1$] when compared with those without MACEs. In contrast, serum levels of CRP were similar in patients with

Table 2 Baseline demographic, clinical, and laboratory features according to the occurrence of major adverse cardiac events

Characteristics	MACEs		P
	Yes (n = 22)	No (n = 178)	
Age (years)	63.4 ± 12.4	62.5 ± 10.2	0.68
Males, *n* (%)	17 (77.3)	133 (74.7)	1.0
Smoking, *n* (%)	6 (27.3)	52 (29.2)	0.81
Diabetes, *n* (%)	10 (45.5)	50 (28.1)	0.12
Hypercholesterolemia, *n* (%)	16 (72.7)	107 (60.1)	0.27
Hypertension, *n* (%)	17 (77.3)	117 (65.7)	0.32
Family history, *n* (%)	6 (27.3)	74 (41.6)	0.27
Acute coronary syndromes, *n* (%)	10 (45.5)	92 (51.7)	0.65
STEMI <3 months, *n* (%)	3 (13.6)	16 (9)	0.44
Previous CABG, *n* (%)	4 (18.2)	16 (9.6)	0.29
Previous PCI, *n* (%)	6 (27.3)	45 (25.3)	0.65
Previous IHD, *n* (%)	18 (81.8)	111 (62.4)	0.11
Ejection fraction	50.2 ± 11.7	56.5 ± 9.1	0.07
Multivessel diseases, *n* (%)	15 (71)	107 (60)	0.36
IIb–IIIa use, *n* (%)	5 (23)	45 (25)	0.83
ECP serum levels (μg/L)	30.5 (13.5–50.1)	12.2 (4.3–31.5)	0.004
ECP levels >11 μg/L	18 (82)	93 (52)	0.007
IgE serum levels (KU/L)	50.3 (26.2–210)	33 (17.3–65)	0.101
CRP serum levels (mg/L)	3.1 (1.6–16)	3 (1.1–8.5)	0.43
Abnormal baseline Troponin T, *n* (%)	6 (29.4)	51 (29)	1
White cell blood count	8.9 ± 2.7	7.8 ± 2.4	0.09
Eosinophil count	0.15 (0.09–0.21)	0.14 (0.08–0.19)	0.8
Neutrophil count	5.4 ± 2.3	4.8 ± 1.7	0.35
Lymphocyte count	2 ± 0.57	2.2 ± 0.7	0.36
Monocyte count	0.49 ± 0.23	0.48 ± 0.23	0.99
Discharge therapy			
Statin, *n* (%)	21 (95)	158 (89)	0.7
Beta-blockers, *n* (%)	16 (75)	144 (81)	0.55
ACE-I or Sartanic, *n* (%)	16 (75)	131 (73.6)	1.0

STEMI, ST-elevation myocardial infarction; IHD, ischaemic heart disease; ECP, eosinophil cationic protein; CRP, C-reactive protein; ACE-I, ACE-inhibitors.

MACEs when compared with those not having MACEs [3 (1.7–15) vs. 3 (1.1–8) mg/L, P = NS]. Furthermore, white blood cell (WBC) count tended to be higher in patients with MACEs when compared with those not having MACEs (8.9 ± 2.7 vs. 7.8 ± 2.4, $P = 0.09$).

Among angiographic and procedural factors, the use of paclitaxel-eluting stent was associated with a higher risk of MACEs when compared with the use of sirolimus-eluting stent ($P = 0.046$). Furthermore, lesions and stents were longer in patients with MACEs, when compared with those without MACEs ($P = 0.018$ and 0.08, respectively). Complex lesions (B2/C) tended to be more frequent in patients with MACEs, when compared with those not having MACEs ($P = 0.12$).

Significant predictors of MACEs at simple Cox regression analysis were ECP serum levels, HR 1.016, 95% CI (1.003–1.030), $P = 0.018$, debiased 95% CI (1.001–1.030), stenosis length, HR 1.027, 95% CI (1.005–1.05), $P = 0.019$, debiased 95% CI (0.98–1.05), stent length, HR 1.020, 95% CI (1.003–1.037), $P = 0.023$, debiased 95% CI (0.997–1.038), and WBC count, HR 1.144, 95% CI (1.001–1.308), $P = 0.048$, debiased 95% CI (1.016–1.350), with previous IHD and use of paclitaxel-eluting stent being of borderline statistical significance (*Table 4*).

The ROC analysis identified the cut-off value of >11 μg/L for ECP [95% CI: >10.3–11.7; sensitivity 82% (debiased 95% CI: 77–89); specificity 52% (debiased 95% CI: 50–56)] and >26 KU/L for IgE levels [95% CI: >22.1–28.3; sensitivity 82% (debiased 95% CI: 78–88); specificity 64% (debiased 95% CI: 60–71)]. Major adverse cardiac event rate was 82% in patients with ECP levels >11 μg/L when compared with 12% in those with ECP levels <11 μg/L ($P = 0.01$). Kaplan–Meier estimates demonstrate that patients with ECP >11 mcg/L had a lower MACE free survival when compared with those having ECP levels <11 mcg/L ($P = 0.008$, *Figure 2*).

Tables 5 and *6* show ECP levels according to clinical, angiographic, and laboratory data. None of these dichotomous variables

Table 3 Baseline angiographic and procedural features according to the occurrence of MACEs

Characteristics	MACEs		P
	Yes ($n = 22$)	No ($n = 204$)	
Culprit vessel, *n* (%)			
LAD	12 (55)	111 (54.5)	0.23
LCX	5 (22.7)	51 (25)	
RCA	3 (13.3)	34 (16.5)	
LM	0 (0)	4 (2)	
SVG	2 (9)	4 (2)	
Stenosis length (mm)	24.5 ± 21.8	17.8 ± 10.3	0.018
Stent type, *n* (%)			0.046
Paclitaxel-eluting stent	16 (72)	102 (50)	
Sirolimus-eluting stent	6 (28)	102 (50)	
Stent number	1.5 ± 0.9	1.5 ± 0.8	0.89
Stent length	18.6 (10–30)	15.4 (11–21)	0.08
B2/C lesions, *n* (%)	16 (72)	108 (53)	0.12
RVD (mm)	2.7 ± 0.51	2.7 ± 0.49	0.89
RVD ≥ 2.75, *n* (%)	8 (36)	90 (44)	0.47
MLD pre (mm)	0.6 ± 0.4	0.6 ± 0.5	0.69
DS % pre	81 ± 11	77 ± 13	0.16
MLD post (mm)	2.37 ± 0.5	2.39 ± 0.45	0.86
DS % post	17.9 ± 10.2	18.5 ± 9	0.80
Acute gain (mm)	1.7 ± 0.7	1.7 ± 0.5	0.91

LAD, left anterior descending; LCX, left circumflex; RCA, right coronary artery; LM, left main; SVG, saphenous vein graft; RVD, reference vessel diameter; MLD, minimal lumen diameter; DS, diameter stenosis.

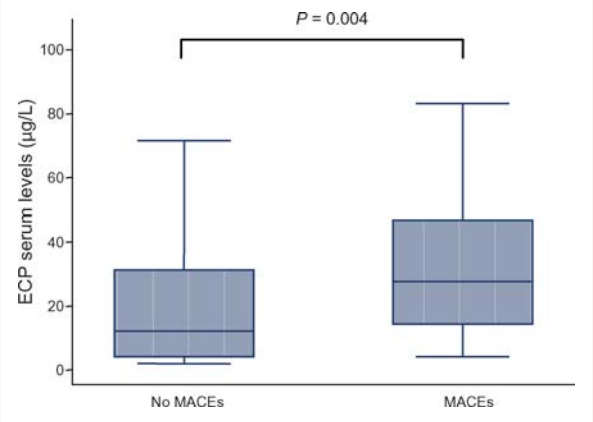

Figure 1 Comparison of eosinophil cationic protein (ECP) serum levels between patients with or without major adverse cardiac event (MACEs). Data are presented as box plots with medians and interquartile range. Patients with MACEs had higher serum levels of ECP compared with those without MACEs ($P = 0.004$).

Table 4 Predictors of major adverse cardiac events at simple Cox regression analysis

	HR (95%CI)	P
Diabetes	1.96 (0.81–4.73)	0.134
Previous IHD	3.30 (0.95–11.54)	0.061
Ejection fraction	0.96 (0.92–1.02)	0.19
ECP levels (μg/L)	1.016 (1.003–1.03)	0.018
IgE levels (KU/L)	0.99 (0.998–1.001)	0.724
WBC count	1.144 (1.001–1.308)	0.048
Stenosis length	1.027 (1.005–1.05)	0.019
Use of PES	2.53 (0.97–6.63)	0.059
Stent length	1.020 (1.003–1.037)	0.023
B2/C lesions	2.28 (0.76–6.78)	0.139

IHD, ischaemic heart disease; ECP, eosinophil cationic protein; WBC, white blood cells; PES, paclitaxel-eluting stent.

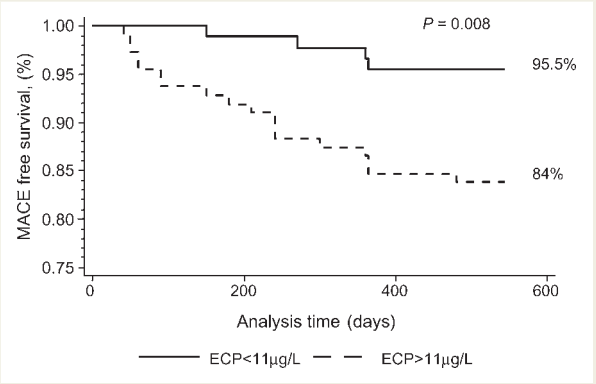

Figure 2 Major adverse cardiac event (MACE) free survival Kaplan–Meier curves according to eosinophil cationic protein (ECP) levels above or below the cut-off value (11 μg/L) identified by ROC curve analysis. Patients having ECP levels >11 μg/L had a worst MACE free survival curve when compared with those having ECP levels <11 μg/L ($P = 0.008$).

was associated significantly with ECP serum levels (*Table 5*). In particular, patients with ACS showed similar ECP serum levels when compared with those with stable angina ($P = 0.40$). No correlation was found between ECP levels and continuous variables (*Table 6*).

Discussion

In this study, we demonstrate for the first time that along with known procedural and angiographic factors, baseline serum levels of ECP, a sensitive marker of eosinophil activation,[22] predict the clinical outcome after implantation of first-generation DES. In contrast, total IgE and CRP serum levels failed to predict the outcome. Of note, being TLR rate prevalent in the composite endpoint in our study when compared with death or MI, our findings should be mainly applied to this endpoint.

Table 5 Eosinophil cationic protein levels according to main dichotomous variables

Variables	Levels	P
Gender		
Male	16 (5–37)	0.14
Female	9 (4–29)	
Hypertension		
Yes	15 (4–31)	0.42
No	15 (6–38)	
Smokers		
Yes	16 (6–39)	0.41
No	16 (5–32)	
Hypercholesterolemia		
Yes	14 (4–31)	0.71
No	15 (5–38)	
Diabetes		
Yes	13 (4–30)	0.23
No	15 (5–38)	
Family history		
Yes	10 (4–31)	0.09
No	18 (6–35)	
Acute coronary syndrome		
Yes	13 (5–33)	0.40
No	17 (5–34)	
Recent STEMI		
Yes	17 (40–5)	0.40
No	17 (40–6)	
Statin therapy		
Yes	15 (5–34)	0.86
No	17 (5–31)	
Beta-blockers therapy		
Yes	15 (5–34)	0.55
No	16 (6–39)	
ACE-I or ARB therapy		
Yes	15 (5–34)	0.74
No	13 (5–30)	
MVD		
Yes	14 (5–32)	0.65
No	15 (5–36)	
Abnormal baseline Troponin T		
Yes	16 (5–31)	0.90
No	11 (4–32)	

ACE-I, ACE-inhibitors; ARB, angiotensin receptor blocker; MVD, multivessel disease; PES, paclitaxel-eluting stent; SES, sirolimus-eluting stent; CRP, C-reactive protein.

Table 6 Correlation of eosinophil cationic protein levels with main continuous variables

Variables	r	P
Age	0.04	0.53
Ejection fraction	−0.015	0.10
IgE levels	0.11	0.13
CRP levels	0.03	0.70
WBC count	−0.12	0.86
Eosinophil count	0.09	0.16
Neutrophil count	−0.35	0.68
Lymphocyte count	0.03	0.71
Monocyte count	−0.05	0.56

CRP, C-reactive protein; WBC, white blood cell count.

Histopathological studies reported the presence of eosinophils associated with BMS in-stent restenosis.[23–25] Of interest, eosinophil infiltrates surrounding stent struts have been described after BMS implantation, but not after balloon angioplasty.[26] Eosinophils appear to be even more involved in DES than in BMS restenosis. Animal studies showed that eosinophil infiltrates develop in 25% of pigs receiving DES.[7] Accordingly, Ribichini *et al.*[8] recently showed a three-fold increase in eosinophil recruitment around paclitaxel-eluting stent when compared with BMS implanted in an animal model.

Drug-eluting stent can promote eosinophil recruitment through different mechanisms. A localized hypersensitivity reaction, in a patient who received a sirolimus-eluting stent, was reported by Virmani *et al.*[27] The authors concluded that polymer-induced inflammation was the cause of eosinophil infiltrates. The drug eluted by the polymer or metal struts, exposed lately after polymer degradation, may as well be involved. Of note, Rittersma *et al.*[28] recently reported a case of eosinophil infiltration in restenotic tissue at the site of a sirolimus-eluting stent which had been implanted for the treatment of a saphenous vein graft in-stent restenosis of a BMS. Eosinophil infiltration was present surrounding the sirolimus-eluting stent but not the BMS, thus suggesting a more important role of either the drug or the polymer rather than metal struts on eosinophil recruitment.

Eosinophils might play an important role not only in restenosis, but also in stent thrombosis. Joner *et al.*[6] reported post-mortem findings in a series of 40 patients who died after stent implantation. The number of eosinophils per strut was higher in DES when compared with BMS. The FDA reported 50 hypersensitivity reactions after DES deployment: post-mortem findings in these patients confirmed intrastent eosinophilic inflammation, thrombosis, and lack of intimal healing.[29] The authors concluded that intrastent hypersensitivity reactions may occur after DES deployment and in some cases may be associated with thrombosis and death, as suggested also by Kounis *et al.*[30]

Of note, eosinophils may induce a pro-thrombotic and inflammatory endothelial phenotype.[31] Furthermore, eosinophil granule proteins have strong pro-thrombotic activity,[32] and deposition of ECP has been observed in vascular necrotic/thrombotic lesion in temporal arteritis,[33] as well as in eosinophilic endomyocardial disease.[34] Finally, platelets may be activated by eosinophil granule proteins.[35]

In light of the potential role of eosinophils in both restenosis and thrombosis of DES, the finding of the present study of an association between basal eosinophil activation and MACEs after DES might be of clinical relevance.

We failed to demonstrate a predictive role of total IgE serum levels on the occurrence of clinical events. Hypersensitivity reactions have been described after DES placement involving IgE-mediated mechanisms and with typical allergic symptoms (urticaria-like rash and serum sickness-like syndromes).[29,36] However, we did not observe such reactions in our study population. Of note, eosinophilic recruitment and activation are not necessarily IgE-mediated, but might be due to a type IV immune reaction mediated by activated T lymphocytes[37] which may be enhanced by basal eosinophil hyper-reactivity. Furthermore, the value of serum total IgE for predicting future allergic reactions has been questioned because of the overlap between allergic and non-allergic patients.[38]

Many studies demonstrated that baseline levels of CRP predict restenosis and clinical outcomes after BMS implantation.[39,40] The local elution of drugs reduces local inflammation, however may offset the higher risk of restenosis associated with high CRP levels. Interestingly, Gaspardone *et al.*[41] have recently demonstrated, in patients undergoing implantation of BMS, DES, or dexamethasone-eluting stent, that despite similar post-procedural elevations of CRP levels, the rate of restenosis was lower in the DES group, thus suggesting that the decreased incidence of stent restenosis observed after DES deployment was unlikely to be related to a decreased systemic inflammatory response, but rather to an increased local resistance to inflammatory mediators. The failure of CRP to predict restenosis after DES in our study is in keeping with the findings of a recent study by Park *et al.*[42] on a large consecutive series of patients undergoing DES implantation, in which tertiles of CRP were not associated with angiographic restenosis and clinical outcomes after 1 year. We acknowledge, however, that no firm conclusion can be drawn about the role of CRP in patients receiving DES. Indeed, two points need to be highlighted: (1) the high frequency of asymptomatic restenosis along with the lack of routine follow-up angiography in our study does not allow to identify all patients with restenosis; (2) the predictive value of CRP mainly applies to long-term mortality, while overall mortality in our study was low.

Stent length was a mild predictor of MACEs after DES implantation in our study in accordance with results obtained in prospective registries,[43] while the use of paclitaxel-eluting stents tended to be associated with higher MACE rate when compared with sirolimus-eluting stents, in accordance with a recent meta-analysis.[44]

We failed to find clinical, angiographic, or laboratory variables associated with higher ECP levels. Thus patient characteristics responsible for different values of ECP cannot be deduced from the result of our study. Eosinophil cationic protein levels may vary according to genetic polymorphisms, as recently suggested by Munthe-Kaas *et al.*,[45] in asthma, with higher levels being associated with more aggressive disease. Eosinophil count has been associated in epidemiological studies to future IHD,[46] and eotaxin, a potent eosinophil chemokine, has been recently associated with an increased coronary atherosclerotic burden.[47] However, we failed to demonstrate differences in ECP levels between ACS and stable angina patients, thus suggesting that eosinophil activation is probably not involved in coronary instability, an issue which deserves future investigations. Finally, the effect of medications on ECP levels should be appropriately evaluated in future prospective studies.

Study limitations

Our study has some limitations. First, given the small event rate, the analysis of individual endpoints is not feasible and we refrained from any multivariable model building because of the high risk of overfitting. Second, we included all comers comprising also patients with ACS who exhibit higher serum levels of inflammatory markers when compared with stable patients. Yet, in our study both ECP and IgE serum levels were similar in patients with stable angina vs. those with ACS. Third, we lack a group of patients with BMS. However, we decided to include patients undergoing DES implantation only, based on recent pathological observations showing that eosinophils are predominantly involved in reaction to DES rather than to BMS.[7,8] Fourth, we did not perform serial assessment of both ECP and IgE levels after stent implantation, which does not allow us to study the contribution of an allergic reaction early after stent implantation. Finally, because of the non-randomized nature of the study, it is difficult to interpret the association of stent type with clinical outcomes.

Conclusion

Recurrence of clinical events after DES implantation is a multifactorial process. Along with procedural and angiographic characteristics, we demonstrate for the first time that enhanced eosinophilic activation at baseline, as assessed by ECP serum levels, is a predictor of MACEs, which in our study is mainly driven by TLR. Further studies are warranted to establish whether in this setting ECP is a risk marker or plays a contributory pathogenetic role.

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