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BIOMEDICAL SCIENCE IN BRIEF

Endothelial progenitor cells mobilisation after percutaneous coronary intervention: a pilot study

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ABSTRACT

Background: The mobilisation process of endothelial progenitor cells (EPC) after stent implantation by percutaneous coronary intervention (PCI) is unclear because the circulating EPC levels are influenced by several pathophysiological factors. The objective was to analyse the kinetics of EPC concentration following elective PCI in patients with stable angina, and its relation with other biomarkers or parameters of cardiovascular function.

Methods: Pilot study in stable angina patients ($n = 30$) for elective PCI and implantation of bare-metal stent (BMS), drug-eluting stent (DES) or EPC-capturing stent (ECS). Samples were taken at baseline, 6 h, 24 h and 6 months after PCI for biochemical analysis and EPC quantification by flow cytometry.

Results: Baseline EPC levels, quantified in peripheral blood, were related with the extent of the coronary lesion and the percentage of stenosis. EPC concentration increased 6 hours after PCI in relation with plasma C-reactive protein concentration and returned to basal levels after 24 hours post-PCI.

Conclusions: Baseline EPC levels are related with the extension of the lesion and stenosis whereas the kinetics of EPC mobilization showed to be related with C-reactive protein concentration. Endothelial activation seems to occur in response to EPC mobilization or vascular damage by PCI.

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Endothelial progenitor cells; elective percutaneous coronary intervention; stent implantation; endothelial progenitor cells mobilization

Percutaneous coronary intervention (PCI) for stent implantation is a common procedure to treat acute and stable coronary artery disease. Circulating endothelial progenitor cells (EPC) derived from bone marrow participate in this process and in the re-endothelialisation of the stents.[1] However, the kinetics of the EPC mobilisation after a PCI for stent implantation is unclear despite previous reports.[2] Even more, the circulating EPC levels are influenced by several factors: circadian rhythms,[3] different concentrations of cytokines or growth factors in blood or pathophysiological status of the patients.[4,5]

In patients with acute coronary syndrome, compared with patients with stable angina following elective coronary angioplasty, a more pronounced increment of EPC counts has been described.[6] However, this increment could be related with higher pro-inflammatory cytokine levels or with an increase in circulating endothelial cells, suggesting an inflammatory response or vascular damage, respectively. In consequence, it is difficult to establish if in an acute PCI, EPC mobilisation responses are to a vascular damage, to an inflammatory response or to an ischaemic process.[7] In this sense, elective interventions seem to cause only a focal endothelial damage, which only cause a very early EPC mobilisation, without any relation with other inflammatory process, although this is controversial.[8,9] Other factors influencing EPC levels are treatment with statins (able to stimulate EPC release from bone marrow,[10]) and levels of advanced glycation end products (AGE), which have been reported to accumulate in tissues and which lead to a decrease in circulating EPC.[11] Taken all together, a pilot study on stable angina patients subjected to elective PCI was conducted in order to determine the crucial parameters to be considered and the size of the population to be included in future prospective studies about the influence of EPC mobilisation after PCI.

Thirty-three consecutive patients with stable angina and/or chronic ischaemic disease documented by imaging techniques and coronary artery disease (coronary artery stenosis >70%) confirmed by catheterisation, were

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Table 1. Clinical parameters of the total population under study and regarding the baseline EPC concentration. Statistic comparison for the groups of high and low EPC levels.

Variable	Total population ($n = 30$)	High baseline EPC (upper median)	Low baseline EPC (under median)	Statistics (P value)
Age (years ± SD)	67.2 ± 11.7	65.9 ± 13.1	68.5 ± 10.3	0.551
Female (%)	36.7	40.0	33.3	0.500
Diabetes mellitus (%)	33.3	26.7	40.0	0.350
Hypertension (%)	76.7	73.3	80.0	0.500
Hyperlipidaemia (%)	80.0	86.7	73.3	0.326
Smokers (%)	46.7	40.0	53.3	0.358
Hemodynamic study				
Multivessel disease (%)	30.0	26.6	33.3	0.189
Disease length, mm	27.2 ± 15.0	30.9 ± 17.7	23.2 ± 10.9	0.172
Stent diameter, mm	3.1 ± 0.4	3.1 ± 0.3	3.0 ± 0.4	0.406
Nº stents	1.50 ± 0.63	1.67 ± 0.72	1.33 ± 0.49	0.150
Contrast volume, mL	135.6 ± 77.1	144.6 ± 65.2	126.7 ± 88.8	0.534
PCI duration, min	9.5 ± 5.9	10.7 ± 4.1	8.4 ± 7.3	0.302
Previous drug treatment				
ASA (%)	100.0	100.0	100.0	NA
Clopidogrel (%)	100.0	100.0	100.0	NA
Heparin, (%)	96.7	93.3	100.0	0.500
Beta-blockers (%)	73.3	73.3	73.3	0.659
RAAS blockers (%)	53.3	33.3	73.3	**0.033**
Oral antidiabetics (%)	26.7	20.0	33.3	0.341
Statins (%)	100.0	100.0	100.0	NA

Abbreviations: ASA: acetylsalicylic acid; PCI: percutaneous coronary intervention; RAAS: renin-angiotensin-aldosterone system.

selected for angioplasty and stent implantation and were eligible for the study. EPC quantification failed in three of these patients; so, 30 patients were finally analysed. They were voluntarily included under informed consent. The exclusion criteria were prior PCI, chronic inflammation, neoplasm, bone marrow diseases, contraindication for dual antiplatelet therapy and treatment with immunosuppressive or non-steroidal anti-inflammatory drugs. The whole study and protocols were approved by the Ethics Committee for Human Studies at Galicia (Spanish region) in accordance to the 1975 Declaration of Helsinki.

A complete medical history, serum biochemistry with Cobas Integra model 700 multichannel analyser (Roche Diagnostics, Indianapolis, USA) and anthropometric data were taken from patients. Electro- and echo-cardiograms as well as haemodynamic study were made prior to the PCI. The diagnosis of diabetes mellitus was based on the latest criteria established by the American Diabetes Association.[12] Hypertension was defined as systolic/diastolic blood pressure >140/90 mm Hg or current use of any antihypertensive medication. Dyslipidaemia was defined by total cholesterol ≥5.69 mmol/l, triglyceride ≥1.69 mmol/l, high-density lipoprotein-cholesterol (HDL-C) <1.03 mmol/l, or current use of anti-hyperlipidemic drugs. Therapeutic strategy and pharmacological treatment were prescribed according to Clinical Practice Guidelines published by the European Society of Cardiology.[13,14]

Peripheral blood samples were taken in EDTA-anticoagulated tubes and plasma were separated by centrifugation (10 min. 2000 × *g*, room temperature) and stored at −40 °C until analysis. Samples were taken at baseline (0 h) between 8:00 and 10:00 am after an overnight fast and at 6, 24 h and 6 months after the PCI.

Creatine kinase-MB, cardiac troponin I (Beckman Coulter Inc., California, USA) and soluble E-selectin (sE-selectin; R&D Systems, Minneapolis, USA) concentrations were measured by ELISA. Estimated glomerular filtration rate (GFR) was used as an indicator of renal function based on the abbreviated modification of diet in renal disease (MDRD) study formula.[15] Cystatin C was measured by a nephelometry kit (N Latex Cystatin C; Dade Behring-Siemens Healthcare España, Madrid, Spain). For hsCRP, samples were processed by automated microparticle immunoassay. AGE were measured by quantitative fluorescence spectroscopy analysis of plasma as previously described.[16] EPC were detected and quantified by flow cytometry (FACS Calibur; Becton Dickinson, New Jersey, USA) of EPC markers (defined as CD45+, CD34+, CD133+, KDR+). Briefly, 200 µL of fresh EDTA-anticoagulated blood samples were stained with four anti-human monoclonal antibodies: allophycocyanin (APC)-conjugated anti-CD34 (eBiosciences, San Diego, USA), carboxy fluorescein (CFS)-conjugated anti-KDR/CD309 (R&D Systems, Minneapolis, USA), phycoerythrin (PE)-conjugated anti-CD133 (eBiosciences, San Diego, USA) and peridinin chlorophyll (perCP)-conjugated anti-CD45 (Immunostep, Salamanca, Spain).

Statistical analysis was performed with SPSS (Statistical Package for the Social Sciences, v. 17.0). The categorical or dichotomous variables were expressed as absolute values and percentages, and were compared with the Pearson X^2 test. Normality was checked with Kolmogorov–Smirnov test. The continuous variables were described as the mean ± standard deviation (SD) when normal or as the median and inter-quartile range for non-parametric data. Comparisons of continuous variables were made with Student *t* test between

Table 2. Laboratory data of the total population under study and regarding the baseline EPC concentration. Statistic comparison for the groups of high and low EPC levels.

Variable	Total population ($n = 30$)	High baseline EPC (upper median)	Low baseline EPC (under median)	Statistics (P value)
Cystatin C, mg/L	0.89 ± 0.27	0.90 ± 029	0.89 ± 0.27	0.984
hsCRP, mg/L	0.66 ± 0.61	0.57 ± 0.75	0.74 ± 0.50	0.531
HbA1c (%)	6.1 ± 1.0	6.0 ± 1.0	6.1 ± 1.0	0.950
Glucose, mg/dL	103.9 ± 20.9	101.5 ± 24.2	106.3 ± 17.5	0.538
GFR (MDRD), mL/min/1.73 m^2	74.1 ± 18.0	71.8 ± 16.6	76.5 ± 19.6	0.484
CK 6 h, U/L	75.7 ± 26.2	84.1 ± 28.6	67.8 ± 21.9	0.095
CK-MB 6 h, ng/mL	1.13 ± 0.52	1.15 ± 0.57	1.10 ± 0.48	0.838
TP-I 6 h, ng/mL	0.02 (0.02–0.08)	0.02 (0.02–0.07)	0.04 (0.02–0.09)	0.290
sE-selectin 6 h, ng/mL	24.4 ± 14.5	25.1 ± 13.8	23.6 ± 15.7	0.784
Fluorescent AGE 6 h, AU	44.6 ± 13.8	49.9 ± 13.2	39.4 ± 12.7	**0.034**
Fluorescent AGE 24 h, AU	45.0 ± 16.0	51.2 ± 16.0	38.9 ± 14.0	**0.033**
Fluorescent AGE 6 months, AU	41.3 ± 18.3	32.5 ± 10.7	54.4 ± 20.2	**0.017**
Leukocytes (cells/μL)	7497.6 ± 2298.3	8118.0 ± 2940.1	6832.9 ± 1071.4	0.131
EPC 6 h, counts/mL	452 (265–792)	603 (283–1156)	291 (129–702)	0.064
EPC 24 h, counts/mL	306 (155–971)	739 (303–1047)	200 (147–340)	**0.050**
EPC 6 months, counts/mL	282 (137–446)	282 (121–446)	289 (124–678)	0.638

Abbreviations: AGE: advanced glycation end products; CK: creatinine kinase; CK-MB: creatinine kinase MB; EPC: endothelial progenitor cells; GFR: glomerular filtration rate; HbA1c: glycated haemoglobin; hsCRP: high-sensitive C reactive protein; TP-I: troponin I.

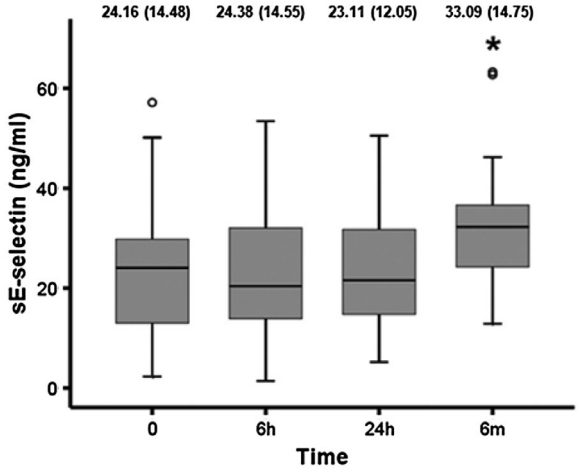

Figure 1. Endothelial activation measured by sE-selectin plasma concentration. Plasma concentration of sE-selectin presented as box plot for each time of sampling of all the study population and as mean values ±SD for each time of sampling. *$P = 0.008$ with respect to other values.

two groups (two tail distribution and equal variances between samples) and with ANOVA test followed by Tukey's test for >2 groups. Non-normal distributed variables were compared with Wilcoxon text for two groups or Kruskal–Wallis test for >2 groups comparison. Correlations between variables were calculated by Pearson's or Spearman's tests according to the normality of the variable. For serial measurement of EPC concentration over the time, variation of EPC counts between basal and final time was considered as summary measure and comparisons were made with two-way ANOVA test. A P value of <0.05 was considered statistically significant.

The usage of bare metal stents (BMS), drug-eluting stents (DES) or EPC capturing stents (ECS) were decided by clinical criteria and the distribution was as follows: 11 BMS, 12 DES and 7 ECS. Mean length of the stents per patient was 19.3 ± 4.7 mm and the mean diameter was 3.1 ± 0.4 mm. No statistical differences were observed between patients regarding the type of stent implanted aside from the incidence of diabetes mellitus (27.3, 58.3 and 0.0% for BMS, DES and ECS, respectively; $P = 0.033$). Baseline characteristics and biochemical measurements of total study population are shown in Tables 1 and 2, respectively. All patients were under double antiaggregant and statins therapy previously to the PCI. The possible relation of baseline EPC levels with the clinical characteristics of patients enrolled is shown in Table 1.

Soluble E-selectin was used as a biomarker of endothelial activation and its baseline level was 24.16 (14.48) ng/mL. After 6 months of stent implantation, the levels of sE-selectin significantly increased in all patients independently of the type of stent implanted (Figure 1). Interestingly, sE-selectin levels 24 h post-PCI were positive related with the levels of EPC before PCI and after 6 h ($r = 0.48$, $P = 0.009$ for baseline EPC and $r = 0.47$, $P = 0.011$ for EPC after 6 h).

Fluorescent AGE levels were measured at baseline and at 6 and 24 h and 6 months post-PCI, with no changes on these levels, showing no influence of PCI on this parameter. However, a negative relation was observed between AGE levels and renal function, as expected. GFR was negatively associated with AGE concentration at baseline ($r = -0.37$, $P = 0.043$) and at 24 h post-PCI ($r = -0.41$, $P = 0.026$). AGE levels at 24 h are also positively related with cystatin C concentration ($r = 0.42$, $P = 0.048$). There was no significant association with plasma levels of glycated haemoglobin, C reactive protein, troponin I or creatine kinase. Importantly, AGE levels at all times up to 24 h after the PCI were positively related with creatine kinase MB concentration in plasma post-PCI ($r = 0.59$, $P = 0.003$ for baseline AGE levels). On the contrary, no relationships were observed between AGE concentration in plasma and the length of the vascular

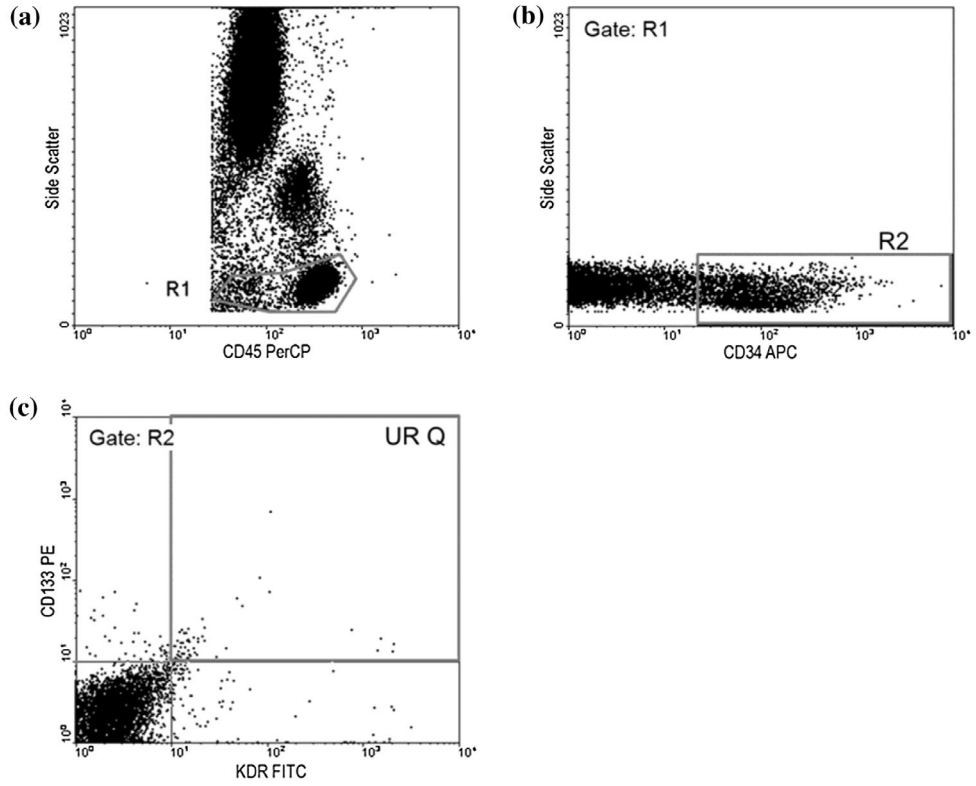

Figure 2. Flow cytometry analysis. Representation of the flow cytometry dot plots showing the gating criteria to count EPC. Panel a shows representative scatter plot for whole blood (hiding the left hand side region of plasma cells and RBC precursors) in which region 1 (R1) gates for lymphocytes CD45+. Panel b shows gate R1 against CD34 marker and region 2 (R2) to select CD34+ cells. Panel c shows gate R2 and the selection of upper right quadrant (UR Q) for CD133+, KDR+ cells.

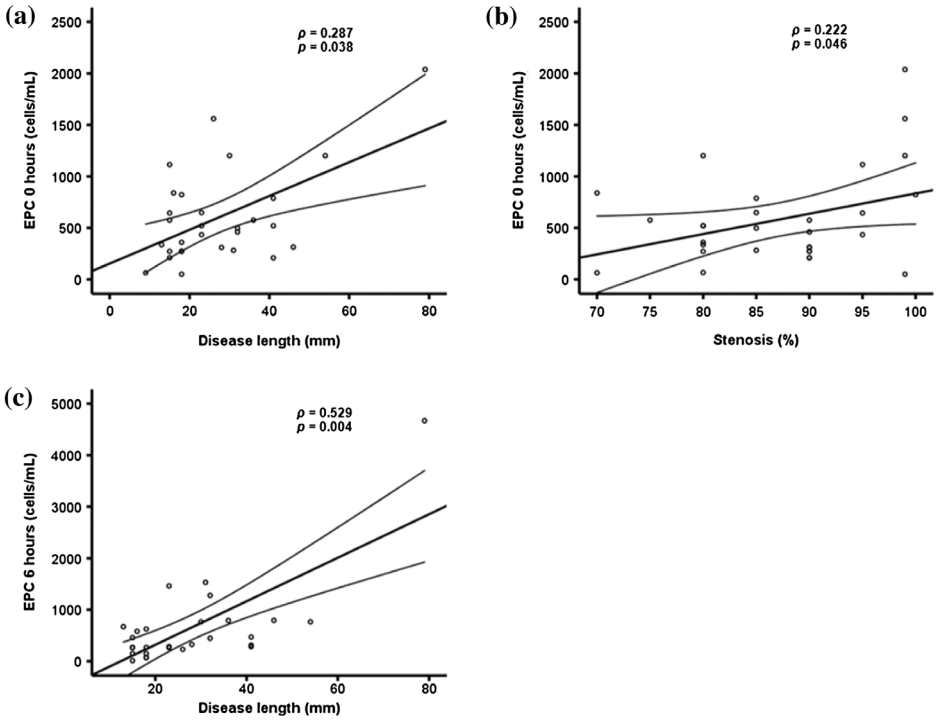

Figure 3. Relation of EPC with coronary artery disease extension. Linear correlation between EPC concentrations at baseline (0 h; panels a and b) or after 6 h post-PCI (c), with total disease length of coronary arteries (a and c) or with percentage of stenosis (b). The straight lines show total adjustment with their confidence intervals (curved lines). ρ = Spearman's rank correlation coefficient.

disease or percentage of stenosis. Baseline AGE levels and EPC counts were positively related, with EPC being significantly higher when AGE were above the median (736.6 ± 467.0 *vs*. 406.5 ± 391.3 EPC/mL for AGE ≥ 45.0 or AGE < 45.0 AU, respectively; $P = 0.047$). Even more, high baseline EPC concentrations were directly related with

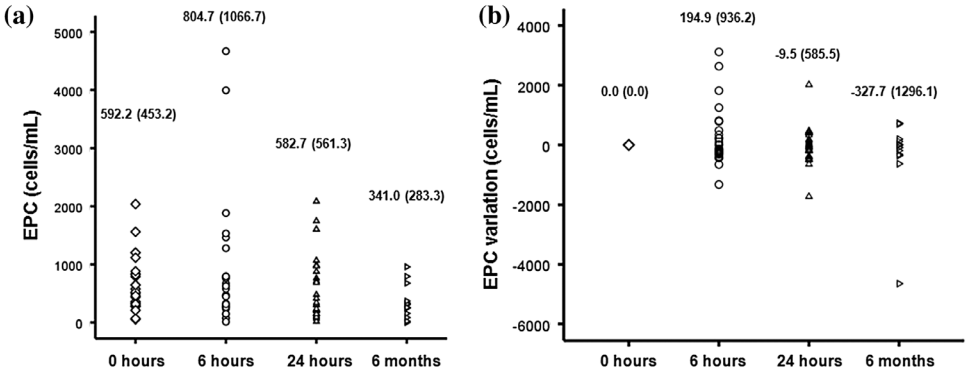

Figure 4. Global EPC kinetics. Representation of the concentration values of EPC in peripheral blood samples at different times regarding to the time after the PCI. Panel a represents EPC concentration in cells/mL for each measurement. Panel b shows concentrations variations with respect to baseline (0 h). Mean values for each time are expressed as mean (SD).

higher AGE levels at 6 and 24 h and with lower AGE at 6 months (Table 2).

Mean value of EPC before the PCI was 582 ± 83 cells/mL (n = 30). Gating criteria to count EPC in flow cytometry analysis is shown in Figure 2. This value was directly related with the total length of the arterial lesion treated (r_s = 0. 28, P = 0. 038) and with the percentage of stenosis (r_s = 0.22, P = 0.046) as shown in Figure 3. Marked variability in the number of EPC ranging from 52 to 2038 cells/mL was noted. After 6 h of the PCI, EPC concentration in peripheral blood slightly increased a 20% (mean value was 694 ± 166 cells/mL). At this time, these values increased their positive relation with the length of the arterial disease (r_s = 0.53, P = 0.004), and even showed a direct relationship with the concentration of hsCRP (r_s = 0.45, P = 0.039). EPC concentration in peripheral blood decreased under the initial levels after 24 h post-PCI and, interestingly, EPC levels went further down after 6 months of PCI (~40% decrease with respect to baseline values; Figure 4). The analysis done by summary measure with total variation of EPC between baseline and six months was not available since some of the data at 6 months were lost as some patients did not complete the study.

This study shows that rapid characterisation and quantitation of EPC can be made in a small volume of peripheral blood counting the population of CD45+, CD34+, CD133+ and KDR+ cells by flow cytometry. Baseline EPC levels were taken always at the same time in the morning since EPC levels are affected by circadian rhythms,[3] from patients of elective PCI all treated with statins. Although the wide range was observed for this value, it showed to be directly related with the extension of the coronary lesion and also with the percentage of stenosis, which suggests that circulating EPC levels are regulated by the inflammatory process affecting coronary arteries in the moment of the intervention. This agrees with the fact that 6 months post-PCI, the EPC levels have decreased in all patients about 40% with respect to baseline values. However, this question is controversial since EPC levels are in a delicate balance between the stimulatory signals for EPC release from the pool of progenitor cells in the bone marrow and their rate of survival, homing and differentiation.[2] Therefore, these points may explain the variability observed in the baseline levels.

Independently of the type of the stent implanted, a slight increase of about 20% on EPC concentration 6 h after the PCI and a total recovery of the baseline levels 24 h after the PCI were observed. The increase observed at 6 h maintains the relationship with the extension of the arterial lesion treated and even with the hsCRP concentration at that time, suggesting an early response of EPC mobilisation to the endothelial damage and the inflammatory process caused by the PCI and the stent implantation. This partially agrees with Padfield et al. that described the same kinetics for circulating CD34+, CD45− cells, but not for CD34+, KDR+ cells.[17] More recently, Gao et al. have found the same EPC kinetics than us during the first 24 h post-PCI for CD133+, CD34+, KDR+ cells.[18] They also described that EPC mobilisation is influenced by the degree of endothelial injury.

Plasma levels of sE-selectin were used as marker of endothelial activation. Although its levels did not change significantly during the 24 h post-PCI, a significative positive relation was observed with the first values of EPC concentration, reinforcing the possible connection between EPC mobilisation and the signalisation from endothelial damage. A marked increase of circulating sE-selectin was observed after 6 months, indicating that endothelial recovery was still an active process at this time, independent of the type of stent implanted.

The increase in EPC levels after stenting related with hsCRP concentration agrees with previously reported data by Garg et al. [8] in non ST segment-elevation myocardial infarction patients. In our study, after 6 months post-PCI, the EPC levels decreased ~40% below the baseline concentration, suggesting the recovery of the vessels. On the contrary, Thomas et al. [9] observed a decrease in the EPC levels after 6 h post-PCI instead of an increase, but also a recovery of the baseline levels after 24 h.

These discrepancies denote the multifactorial dependency of EPC mobilisation and the kinetics in blood.

There was no relation between circulating biomarkers of myocardial injury (creatine kinase and its isoenzyme MB and troponin I) and EPC levels. This is probably due to the fact that patients for elective PCI are in a clinically stable situation and that PCI is not a myocardial damaging process.[19]

Although tissue and serum AGE levels have been inversed related with circulating EPC levels,[11,20] we found a direct relation between high plasma AGE concentrations and high EPC counts at baseline. Fluorescent AGE levels did not show any dependence neither on the PCI process or the type of stent implanted nor on EPC mobilisation after PCI. However, independent of these processes, our data suggest partial dependence of EPC circulating levels on the degree of advanced glycation. Otherwise, AGE levels were strongly related with creatine kinase-MB at 6 h reflecting the positive relation of these adducts with cardiovascular injury.

A major limitation of this study is the small sample size because of the difficulty to get a high number of blood samples per patient (4 at different hours or months), the difficulty and time needed for the EPC counts (immunological staining and flow cytometry). Second, our study was made only in patients with stable angina and/or chronic ischaemic disease subjected to elective PCI, so, the results can be different in patients with myocardial infarction or under an acute coronary syndrome. Thirdly, sampling occurred at only four time points, hence it is not possible to know when the exact peak/decline in these markers would have occurred.

This work represents an advance in biomedical science because, using a simple method for EPC quantification, it finds the positive relationships between EPC and coronary lesion extension and between EPC mobilisation, C-reactive protein and endothelial activation. Further investigations are needed to evaluate clinical prognostic value of circulating EPCs.

Conflict of interest

Nothing to declare.

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