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Impact of a cardiac rehabilitation program and inflammatory state on endothelial progenitor cells in acute coronary syndrome patients

Francesca Cesari [a,*], Rossella Marcucci [a], Anna Maria Gori [a,c], Costanza Burgisser [b], Sara Francini [b], Francesco Sofi [a,c], Gian Franco Gensini [a,c], Rosanna Abbate [a], Francesco Fattirolli [b]

[a] Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy
[b] Cardiac Rehabilitation Unit, University of Florence, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy
[c] Centro S. Maria agli Ulivi, Fondazione Don Carlo Gnocchi Italia, Florence, Italy

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ABSTRACT

Background: Among the benefits of a cardiac rehabilitation (CR) program for patients after an acute coronary syndrome (ACS) is the mobilization of endothelial progenitor cells (EPCs). However not all patients respond to CR with an increase of EPC. We performed this study to identify the characteristics of patients who will not benefit from an increase of EPCs at the end of a CR program.

Methods: 112 ACS patients were admitted to a four-week CR program. EPCs, high sensitivity C-reactive protein (hsCRP) and NT-ProBNP levels were determined at the beginning (T1) and at the end (T2) of the CR program. All patients performed a cardiopulmonary exercise test at T1 and at T2. EPCs were defined as CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+. hsCRP and NT-ProBNP were measured by nephelometric and immunometric method, respectively.

Results: At T2, we observed a significant increase of EPCs ($p=0.001$), VO_2 peak, Watt max HDL-cholesterol ($p<0.0001$) and a significant decrease ($p<0.001$) of hsCRP and NT-ProBNP, triglycerides, HbA1c, systolic blood pressure and waist circumference. Variations of VO_2 peak were significantly correlated with the variations of EPCs. Patients with increased EPCs showed significantly ($p=0.01$) lower baseline levels of CRP and higher basal Watt max ($p=0.04$). In a multivariate logistic regression analysis, the lowest tertile of baseline hsCRP significantly affected the likelihood of having an increase of EPCs at the end of the CR program.

Conclusions: A CR program determines an increase of EPCs with a decrease of CRP and NT-ProBNP. A different trend for EPCs can be detected among patients correlated to CRP levels and exercise tolerance.

1. Introduction

The role of cardiac rehabilitation (CR) and secondary prevention programs after acute coronary syndrome (ACS) are well established: basically interventions are designed to optimize cardiovascular (CV) risk reduction, encourage healthy lifestyle modifications and compliance, reduce CV disability, and promote an active lifestyle [1–3].

The most substantial evidence-based benefits of CR include an improvement in exercise tolerance. The benefits of an appropriately prescribed and implemented exercise training (ET) program include enhancement of functional capacity, with a consequent increase in activity threshold before the onset of ischemia and reduction of symptoms; enabling patients to return to work and recreational activities [4–6]. The mechanisms by which physical exercise confers such significant protection range from the reduction of risk factors to the improvement of endothelial function [7,8]. In recent years, increasing evidence has shown that physical activity (PA) can also modulate levels and function of circulating progenitor cells (CPCs) from the bone marrow, and in particular of the subset named endothelial progenitor cells (EPCs) which enhance angiogenesis, promote vascular repair, and improve endothelial function [9–12]. This may represent an additional important beneficial outcome of physical exercise, probably mediated by a shear stress-induced up-regulation of endothelial nitric oxide synthase and by the subsequent increase in nitric oxide bioavailability [13]. Another possible beneficial effect of PA is the attenuation of inflammatory mediators; it has been previously reported that physical (ET) induces an improvement of the inflammatory state, with a decrease of pro-inflammatory markers such as interleukin-6 and C-reactive protein (CRP) [14,15].

On this subject we recently reported how the improvement of exercise capacity after a short period of CR intervention is also associated with modifications of EPCs' number in cardiac surgery patients [16]. Despite this, however, not all the patients undergoing a CR program show an equal response to ET after an acute event in terms of an increase of EPCs' number [16,17]. The lack of increase of EPCs following

* Corresponding author at: Department of Medical and Surgical Critical Care, Thrombosis Centre, University of Florence, Azienda Ospedaliero-Universitaria Careggi, Viale Morgagni 85, 50134 Florence, Italy. Tel.: +39 055 7949420; fax: +39 055 7949418.
E-mail address: francesca.cesari@gmail.com (F. Cesari).

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a CR program may determine a significant worsening of the CV risk profile of these patients.

We conducted this study to evaluate in a population of ACS patients who underwent percutaneous coronary intervention, the effect of a CR program on levels of CPCs, EPCs and inflammatory markers, the relationship between the improvement of exercise capacity and the variations of EPCs and CPCs, and most importantly, indicators of a non-increase of EPCs' number at the end of a CR program.

2. Materials and methods

2.1. Study population

The initial study population consisted of 117 patients who were admitted to the Cardiac Rehabilitation Unit of the University Hospital of Careggi after a hospitalization for ACS from May 2007 to April 2009. Five patients dropped out the study: 3 (1 M/2 F) for recurrence of angina, 1 (male) to undergo elective surgery for abdominal aortic aneurysm and 1 refused to attend the CR protocol.

The final study population consisted of 112 patients (92 male; 20 female, mean age 58.2 ± 9.5). In the sample 83 patients (74.1%) were affected by STEMI (ST Elevation Myocardial Infarction) and 29 (25.9%) by NSTEMI (No ST Elevation Myocardial Infarction). All patients underwent coronary angiography and primary percutaneous coronary revascularisation at the Department of Heart and Vessels of the same Hospital. 79 (70.5%) patients were treated with Drug Eluting Stent (DES), 29 (25.9%) with Bare Metal Stent (BMS) and 4 (3.6%) by Plain-Old Balloon Angioplasty (POBA).

Exclusion criteria were left ventricular ejection fraction <30%, age >75 years, presence of any medical conditions that would make exercise unsafe (unstable angina, sustained ventricular arrhythmias, symptomatic COPD, uncontrolled arterial hypertension, uncontrolled diabetes, hyperthyroidism) or any functional conditions that would limit the ET program.

All patients were treated from day one with statins (44.6% simvastatin, mean dosage: 27 ± 10 mg/day; 37.5% atorvastatin, mean dosage: 34 ± 12 mg/day; 17.9% rosuvastatin mean dosage: 15 ± 8 mg/day) and were included in the study at least 30 days after the cardiac event. This timing was established on the basis of previous data documenting an effect of the acute phase and of revascularization procedure on EPCs' number and the other biomarkers analyzed [18,19].

Moreover, all patients were on dual antiplatelet therapy with aspirin (100 or 325 mg) and clopidogrel (75 mg).

Current smoking status was determined at the time of blood collection. Subjects were classified as having hypertension according to the guidelines of European Society of Hypertension/European Society of Cardiology [20]. Diabetic subjects were defined in agreement with the American Diabetes Association [21] or on the basis of self-report data (if confirmed by medication or chart review). Dyslipidemia was defined according to the Third report of the National Cholesterol Education Program (NCEP-III) or if they reported taking antidyslipidemic drugs, as verified by a physician [22]. A positive family history was defined as the presence of at least one first-degree relative who had developed coronary artery disease before the age of 55 years for men and 65 years for women. Body mass index (BMI) was calculated as weight (kg)/height (m^2) and obesity was defined as BMI ≥30. All subjects gave informed consent; the study complies with the Declaration of Helsinki and was approved by the local ethic committee. The authors of this manuscript have certified that they comply with the principles of ethical publishing in the International Journal of Cardiology.

2.2. Cardiopulmonary exercise test

A baseline cardiopulmonary exercise testing (CPET) was performed as part of the patient's routine test to assess functional capacity and to tailor the subsequent ET,. CPET was performed on an electrically breaked cycle ergometer with a progressively increasing work rate, controlled by Stress Test System Esaote Biomedica, which recorded a 12-lead exercise ECG during the test. The exercise protocol was determined by the age and general fitness of the patient, and varied from 10 Watt work load increments every 1 min to 25 W every 2 min.

Breath-by-breath measurements of oxygen uptake, carbon dioxide production, and respiratory flow and volume parameters were obtained by applying a Hans Rudolph mask, connected to a pneumotach device. A *CPX Ultima* metabolic monitor was used to perform gas exchange analysis, linked into the *BreezeSuiteTM* software package (Medical Graphics Corporation). Oxygen, carbon dioxide and flow sensors were calibrated immediately before each test. Arterial pressure was measured manually every 1 or 2 min by cuff sphygmomanometer. The reasons for test termination were those established by international guidelines [23].

Exercise capacity was expressed as maximal workload (Watt); peak oxygen uptake (VO_2 peak) was averaged over the last 30 s of exercise, and expressed relative to body weight (ml/kg/min). CPET was repeated at the end of CR program.

2.3. Cardiac rehabilitation and training protocol

All patients took part in a 30 days CR program consisting of optimal therapeutic management according to available guidelines, serial clinical and instrumental examinations and exercise program [24]. In detail, the training protocol consisted of supervised exercise sessions (mean: 14 ± 4.7), 3 days/week of endurance training on a cycle-ergometer (5-minute warm-up, 30-minute training at constant workload and 5-minute cool-down). In each session, ECG was monitored by telemetry. Exercise intensity was determined individually at 60–70% of VO_2 level obtained during baseline symptom-limited CPET. Patients received CV risk factor management counseling twice a week and were invited to join with family members a support group.

2.4. Blood collection

Blood samples were obtained in the morning after an overnight fasting at two time points for each patient: time 1 (T1), at the beginning of the CR program (30 ± 5 days after the acute event) and time 2 (T2) at the end of the CR program. Samples at T2 were obtained at least 48 h after the last program of exercise in the CR protocols. Blood samples were collected from the antecubital vein into evacuated plastic tubes (Vacutainer) anticoagulated with ethylenediaminotetracetate (EDTA) 0.17 mol/l for obtaining plasma samples for CPCs' and EPCs' evaluation and with no anticoagulant for obtaining sera samples for hsCRP and NT-ProBNP evaluation. The sera samples were centrifuged at 2000 *g* for 10 min a 4 °C and then stored in aliquots at −80 °C until analysis.

2.5. Flow cytometric analysis

CPCs and EPCs' number were assessed contemporarily using flow cytometry, as previously described [16,25,26].

Briefly, 200 μl of peripheral venous blood were incubated for 20 min in the dark with:

- fluoresceine isothiocyanate (FITC)-labeled monoclonal antibodies against human CD34 (BD Pharmingen, San Diego, California, USA);
- allophycocyanin (APC)-labeled monoclonal antibodies against human AC133 (Miltenyi Biotec, Bergisch Gladbach, Germany);
- Phycoerythrin (PE)-labeled monoclonal antibodies against human VEGFR2-KDR (R&D Systems Inc, Minneapolis, USA);
- allophycocyanin-cyanin7 (APC-Cy7)-labeled monoclonal antibodies against human CD45 (Becton Dickinson, San Jose, USA); and
- LDS751, a nucleic acid dye (Molecular Probes, Invitrogen, Eugene, Oregon, USA).

Mouse isotype-identical antibodies served as controls (Becton Dickinson, San Jose, CA, USA). Red blood cells and platelets were subsequently lysed by NH_4Cl lysing solution (Autolyse solution; BioSource International, Camarillo, USA). For analysis, 300,000 cells within the leukocyte gate were acquired using a FACSCanto analyzer (Becton Dickinson, San Jose, USA) and data were processed using BD FacsDiva software. Circulating EPCs were identified through their expression of CD34, KDR, and CD133 and were considered as endothelial progenitor cells CD34+/KDR+, CD133+/KDR+ and CD34+/CD133+/KDR+.

By using a modification of the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines [27] CPCs were defined as cells forming a cluster with low side scatter and low-to-intermediated CD45 staining and positive for CD34+, CD133+ and CD34+/CD133+.

2.6. High sensitivity C-reactive protein (hsCRP) determination

hsCRP was assessed with a high-sensitivity assay on a BN II nephelometer (Dade Behring, Marburg, Germany).

2.7. NT-pro-BNP determination

NT-pro-BNP was measured with a chemiluminescent immunoassay kit (Roche Diagnostic Laboratory, Indianapolis, IN, USA) on an Elecsys 2010 analyzer.

2.8. Statistical analysis

Statistical analysis was performed using the SPSS (Statistical Package for Social Sciences, Chicago, IL, USA) software for Windows (Version 18.0). Values are presented as median and range or by mean and standard deviation as appropriate.

The Chi-square test was used to identify statistically significant differences between categorical variable. The Mann–Whitney test for unpaired data was used for comparison between groups. The Wilcoxon test for related data was used to evaluate differences between two time-points. The Spearman's test was used to identify significant correlations between two numerical variables.

In order to analyze variables related to EPCs' improvement we divided our population into patients who reported an increase of at least one type of EPCs (group A) at the end of the CR period and those who reported a decrease or no increase of EPCs (group B). A multiple logistic regression analysis was used to test the independent association between CRP tertiles [1st tertile <2.2 mg/l, 2nd tertile 2.2–5.1 mg/l, 3rd tertile >5.1 mg/l] and the likelihood of EPCs' increase. All odds ratios (OR) are given with their 95% confidence interval. Moreover, to test EPCs' number in relation to exercise capacity we calculated the delta (Δ = end-onset of the CR) of the VO_2 max (ΔVO_2 max), and of EPCs (Δ CD34+/KDR+; ΔCD133+/KDR+; ΔCD34+/CD133+/KDR+). $P<0.05$ was considered to be statistically significant.