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Left ventricular mass and progenitor cells in chronic heart failure patients

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Abstract The aim of the study was to evaluate the association between circulating (CPCs) and endothelial (EPCs) progenitor cells and left ventricular (LV) remodeling in chronic heart failure (HF). 85 HF patients, ranging 29–89 years, 83.5 % males, 45.9 % ischemic, NYHA functional class II–IV, with a LV ejection fraction ≤40 % were studied. LV ejection fraction, LV end-diastolic and end-systolic (LVESV) volumes, LV mass and tricuspid annular plane systolic excursion (TAPSE) were evaluated, and, when indicated, indexed for body surface area (BSA). CPCs and EPCs number was assessed using flow cytometry. CPCs were defined as CD34+, CD133+ and CD34+/CD133+. EPCs, identified through their expression of KDR, were defined as CD34+/KDR+, CD133+/KDR+ and CD34+/CD133+/KDR+. All EPCs were negatively related to LVESV/BSA ($r = -0.24$, $p = 0.02$ for all EPC's populations), and to LVmass/BSA (CD34+KDR+; $r = -0.30$, $p = 0.005$; CD133+KDR+; $r = -0.31$, $p = 0.004$; CD34+CD133+KDR+; $r = -0.29$, $p = 0.007$). No differences in EPCs levels in relation to cardiovascular risk factors, medications, etiology, age or gender were observed. CPCs number was higher in women, and lower in ischemic patients. In logistic regression analyses, the low EPCs' number was associated with an increased likelihood of abnormal LVmass/BSA. CPCs proved to be higher and EPCs lower in patients with severely abnormal LVmass/BSA (gr/m^2, ≥122 in women and ≥149 in men). Our results suggest a correlation between LV remodeling and progenitor cells. This is noteworthy considering that it has been suggested that bone marrow-derived EPCs participate in cardiac regeneration and function recovery in the setting of progressive HF.

Keywords Progenitor cells · Heart failure · Ventricular remodeling · Ventricular mass

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Introduction

It has been reported that persistent mobilization of bone marrow progenitor cells correlates with favorable left ventricular (LV) remodeling, as evidenced by prevention of LV dilatation and enhanced contractile recovery in patients with acute myocardial infarction [1, 2]. However, data on the association between mobilization of progenitor cells and LV remodeling in chronic heart failure (HF) patients are few. Among the scant data about this issue, it has been previously suggested that bone marrow-derived endothelial progenitor cells participate in cardiac regeneration and function recovery in the setting of progressive HF [3, 4]. The aim of the present study was to better analyze the relationship between blood levels of progenitor cells and LV remodeling in HF patients. For this purpose, we estimated both endothelial (EPCs) and circulating (CPCs) progenitor cells. From a biological point of view, EPCs and CPCs likely represent different progenitor cell phenotypes with different biological properties. The EPCs circulating pool represents a population of more mature cells that are

just "committed" to differentiate into endothelial cells, able to participate mainly in the processes of re-endothelialization and revascularization. CPCs represent a more heterogeneous and undifferentiated cell population, able to evolve in different cell types.

Materials and methods

Study population

Our population consisted of 85 consecutive chronic HF out-patients, referred to our Heart Failure service in the period 2008–2011. All participants gave informed written consent, and the study protocol was approved by the local ethics committee. All clinical data, echocardiographic evaluation and blood sample collection were performed for each patient on the day of recruitment visit. The median age was 67 years (range 29–89); 71 (83.5 %) patients were men. The New York Heart Association (NYHA) functional class was II–IV. All participants had a LV ejection fraction (LVEF) $\leq$40 %. The median QRS duration was 140 ms (range 80–200 ms). Fifty-seven out of 85 patients (67.1 %) showed narrow QRS ($\leq$120 ms), remaining patients had a QRS >120 ms, and a left bundle branch block morphology. The QRS duration was measured from the surface ECG using the widest QRS complex. The QRS duration was scored by two independent observers who were blinded to all other patient data. All patients had to be clinically stable for a period of at least 3 months preceding the enrollment. All patients presented in a normal sinus rhythm. HF etiology was ischemic in 39 patients (45.9 %) and non-ischemic in 46 patients (54.1 %). Forty-eight patients (56.5 %) were hypertensive, 34 (40 %) dyslipidemic, 16 (18.8 %) diabetic and 47 (55.3 %) were smokers.

Echocardiographic protocol

Echocardiographic studies were performed using a high-quality echocardiograph (Vivid 9, GE, USA) equipped with a 5S probe. All measurements were performed by the same expert cardiologist according to the American Society of Echocardiography recommendations, [5] and parameters, when necessary, were indexed by body surface area (BSA). The following echo M-mode, bi-dimensional and pulsed Doppler parameters were evaluated: LV end-diastolic and end-systolic volumes (LVEDV and LVESV, respectively); LVEF estimated with the biplane Simpson's method. Tricuspid annular plane systolic excursion (TAPSE) was estimated by two-dimensional echo-guided M-mode recordings from the apical 4-chamber view, the cursor placed at the free wall side of the tricuspid annulus. Relative wall thickness was defined as (posterior wall thickness + interventricular septal wall thickness)/internal diameter, and LV mass was calculated according to the formula of Devereux [6–8]. Concentricity was defined as LV mass divided by LVEDV. LVmass/BSA (gr/m^2) was considered severely abnormal when, as previously indicated, [7] its value was $\geq$122 in women and $\geq$149 in men.

Flow cytometric analysis of CPCs and EPCs

CPCs and EPCs' number was assessed contemporarily using flow cytometry, as previously described [9, 10]. Briefly, 200 µl of peripheral venous blood was incubated for 20 min in the dark with:

- fluorescein 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-cyanine7 (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 NH4Cl 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+.

Using a modification of the International Society of Hematotherapy and Graft Engineering (ISHAGE) guidelines, [11] 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+.

NT-proBNP determination

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

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 variables. The Mann–Whitney test for unpaired data was used for comparisons between groups. The Spearman's test was used to identify significant correlations between numerical variables. A multiple logistic regression analysis was used to test the independent association between EPCs number and the likelihood of an abnormal LVmass/BSA. All odds ratios (OR) are given with their 95 % confidence interval.

Results

Demographic and clinical characteristics of the study population are reported in Table 1. All EPC's populations were negatively and significantly related with LVESV/BSA ($r = -0.24$, $p = 0.02$ for all EPC's populations), and a tendency towards significance was observed for LVEDV/BSA ($r = -0.19$, $p = 0.08$ all EPC's populations). Moreover all EPCs' populations were negatively and significantly related to LVmass/BSA (CD34+KDR+; $r = -0.30$, $p = 0.005$; CD133+KDR+; $r = -0.31$, $p = 0.004$; CD34+CD133+KDR+; $r = -0.29$, $p = 0.007$). In a logistic regression analysis, the low EPCs' number was associated with an increased likelihood of abnormal LVmass/BSA, even after adjusting for age, gender, cardiovascular risk factors and statin use [OR 95 % CI CD34+KDR+ 1.12 (1.02–1.22), $p = 0.01$; CD133+KDR+ 1.13 (1.03–1.25), $p = 0.01$; CD34+CD133+KDR+ 1.14 (1.03–1.27), $p = 0.01$]. No significant correlations were observed between CPCs and echocardiographic parameters. To further analyze the behavior of progenitor cells, we compared patients with or without severely abnormal LVmass/BSA ($\geq$122 gr/m^2 in women and $\geq$149 gr/m^2 in men). CPCs proved to be significantly higher and EPCs significantly lower in patients with severely abnormal LVmass/BSA (Table 2). There was no significant correlation between EPCs and CPCs levels. According to left ventricular mass/BSA cutoff, significant differences in LVESV/BSA, LVEDV/BSA, concentricity and ischemic etiology were detected (Table 3), while age, gender, NYHA class and ejection fraction did not differ between patients with and without severely abnormal LVmass/BSA. Evaluating cardiovascular risk factors and therapies, a significantly higher proportion of diabetic patients and of statin use was observed in patients without severely abnormal LVmass/BSA with respect to patients with an abnormal LVmass/BSA [diabetes: 10/26 (38.5 %) vs. 6/59 (10.2 %), $p = 0.002$; statin use 16/26 (61.5 %) vs. 23/59 (38.9 %), $p = 0.04$.] No significant differences of

Table 1 Clinical characteristics of patients investigated

	Overall group ($n = 85$)
Age, years[a]	67 (29–89)
Male gender, *n*(%)	71 (83.5)
NYHA class[a]	3 (2–4)
Diabetes, *n* (%)	16 (18.8)
Smoking, *n* (%)	47 (55.3)
Hypertension, *n* (%)	48 (56.5)
Dyslipidemia, *n*(%)	34 (40.0)
ACE-inhibitors, *n* (%)	55 (64.7)
β-Blockers, *n* (%)	67 (78.8)
Allopurinol, *n* (%)	16 (18.8)
Statins, *n* (%)	39 (45.9)
Diuretics, *n* (%)	61 (71.8)
Antialdosteronics, *n* (%)	37 (43.5)
Angiotensin receptor blockers, *n* (%)	14 (16.5)
Antiarrhythmics, *n* (%)	14 (16.5)
Digoxin, *n* (%)	7 (8.2)
Ischemic etiology, *n* (%)	39 (45.9)
LBBB QRS morphology, *n* (%)	28 (32.9)
QRS duration[a]	140 (80–200)
Left ventricular mass/BSA (g/m^2)[a]	161.36 (88.16–763.70)

[a] Values are expressed as median and (range)

Table 2 Circulating progenitor cells' (CPCs) and endothelial progenitor cells' (EPCs) number in patients with (cutoff: women $\geq$122 g/m^2; men $\geq$149 g/m^2) or without severely abnormal left ventricular mass/BSA (LVmass/BSA)

	Severely abnormal LVmass/BSA ($n = 59$)	No severely abnormal LVmass/BSA ($n = 26$)	*p*
CPCs			
CD34+[a] (cells/10^{6}events)	270 (80–743)	211(97–1129)	***0.04***
CD133+[a] (cells/10^{6}events)	243 (57–690)	183 (47–1139)	***0.03***
CD34+CD133+[a] (cells/10^{6}events)	243 (57–690)	171(47–1129)	***0.03***
EPCs			
CD34+KDR+[a] (cells/10^{6}events)	3 (0–23)	10 (0–33)	***0.009***
CD133+KDR+[a] (cells/10^{6}events)	3 (0–20)	10 (0–33)	***0.009***
CD34+CD133+KDR+[a] (cells/10^{6}events)	3 (0–17)	10 (0–27)	***0.013***

[a] Values are expressed as median and (range)

Table 3 Differences in age, gender, etiology, echocardiography parameters, NYHA class and QRS duration between patients with (cutoff: women ≥122 g/m^2; men ≥149 g/m^2) or without severely abnormal left ventricular mass/BSA (LVmass/BSA)

	Severely abnormal LVmass/BSA ($n = 59$)	No severely abnormal LVmass/BSA ($n = 26$)	p
Males, n(%)	47 (79.6)	24 (92.3)	*0.20*
Age (years)[a]	67 (29–85)	66 (49–89)	*0.20*
Ischemic etiology, n(%)	24 (40.7)	15 (57.7)	***0.04***
QRS duration (ms)[a]	140 (80–200)	120 (80–200)	*0.06*
LVESV (ml/m^2)[a]	73.53 (38.38–154.22)	57.90 (32.28–103.49)	***0.007***
LVEDV (ml/m^2)[a]	101.68 (50.92–204.82)	80.07 (49.74–143.60)	***0.002***
Concentricity[a]	1.71 (0.98–5.42)	1.48 (0.89–2.12)	***<0.0001***
TAPSE (mm)[a]	20 (12–29)	19 (11–23)	*0.08*
LVEF (%)[a]	30 (14–40)	29.5 (21–38)	*0.99*
NYHA class[a]	3 (2–4)	3 (2–4)	*0.57*

[a] Values are expressed as median and (range)

EPC levels in relation to cardiovascular risk factors, medications, etiology, age or gender were observed (data not shown). On the contrary, CPCs' number was significantly higher in women with respect to men [CD34+ 288 (150–693) vs. 230 (47–1129) cells/106 events, $p = 0.04$; CD34+/CD133+ 267 (150–690) vs. 207 (47–1139) cells/106 events, $p = 0.02$; CD133+ 267 (150–690) vs. 207 (47–1129) cells/106 events, $p = 0.02$] and significantly lower in patients with an ischemic etiology [CD34+ 182 (80–497) vs. 258 (47–743) cells/106 events, $p = 0.04$; CD34+/CD133+ 173 (57–493) vs. 233 (47–690) cells/106 events, $p = 0.05$; CD133+ 173(57–497) vs. 240 (47–690) cells/106 events, $p = 0.04$]. In addition, a significant and negative correlation between CPCs' number and age was observed ($r = -0.23$, $p = 0.03$ for all CPC's populations). NT-ProBNP levels were significantly and positively related with age ($r = 0.25$, $p = 0.02$), and, albeit not significantly, inversely related with CD34+KDR+ EPCs ($r = -0.19$, $p = 0.07$).

Discussion

This study evaluated the relationship between the degree of ventricular remodeling and blood levels of progenitor cells, measuring both EPCs and CPCs, in chronic HF patients. All EPCs populations were negatively related to LVESV/BSA and LVmass/BSA, and the presence of a lower number of EPCs was associated with an increased likelihood of abnormal LV mass/BSA. These data suggest a correlation between higher number of circulating EPCs and a less severe pathological state, with more preserved LV function and a lower degree of ventricular remodeling. A possible explanation for these findings, specifically for the relationship between LVESV/BSA or LVmass/BSA and EPCs, can be inferred from the role of afterload in determining ventricular end-systolic volume [12], with increased afterload inducing pathological cardiac hypertrophy [13]. A possible role of afterload in influencing progenitor cells might be considered.

We then evaluated the correlation between LV mass and progenitor cells, dividing patients according to a pre-established cutoff for indexed LV mass, indicating the presence or absence of severely abnormal values [7]. All CPCs populations proved to be higher, and all EPCs lower, in patients with severely abnormal LVmass/BSA. The existence of a relationship between LV mass and progenitor cells is noteworthy, considering that LV hypertrophy is a frequent consequence of hypertension, and is thought to be involved in the subsequent occurrence of congestive heart failure [14, 15]. A relationship between EPCs and LV remodeling had been previously reported only in patients with acute myocardial infarction, and never in chronic HF patients. Previous clinical studies [1, 2] show a reduced mobilization of progenitor cells in the acute phase of myocardial infarction as well as its recovery over 1-year follow-up; a greater reduction of mobilization is associated with more significant impairment of LVEF and greater infarct size. The only study pertinent to our results was performed in 22 chronic HF patients scheduled for cardiac resynchronization therapy [16]. In this study, no relationship between progenitor cells and LV remodeling was observed; however, it was conducted measuring only CPCs, and not EPCs. Two studies [17, 18] reported a reduced number of EPCs in hypertensive patients, but both were conducted without evaluating LV mass and analyzing the presence of hypertrophy only by electrocardiography, whose sensitivity in detecting the presence of cardiac hypertrophy is only 6 to 20 % [19, 20]. In both the cases, the populations under study did not include HF patients. To interpret the different behavior of CPCs and EPCs in relation to left ventricular mass, we should consider that CPCs and EPCs represent different progenitor cells phenotypes with probably a different biological meaning. The CPCs' population contains stem cells of various lineages, and is a reservoir of haematopoietic, endothelial, neurological and cardiac cells. This "multipotency" of CPCs with respect to EPCs, (which are just committed to the endothelial lineage), and the capacity to shift into a particular type of cells in relation to the stimulus, can explain the differences between the two types of cells. The absence

of a correlation between the two types of cells, shown in the results, might suggest an independent role for these cells populations, further supporting their different behavior. In recent years, it has been shown that the most important mechanism by which progenitor cells promote angiogenesis and vasculogenesis is their paracrine function [21, 22]. As progenitor cells become committed to the endothelial lineage, they lose CD133 and acquire KDR; this transition is associated with phenotypic properties more closely related to a mature endothelium, with a possibly different paracrine activity [23]. EPCs possess more pronounced angiogenic properties than CPCs [23], thus a low number of EPCs may be associated with a compromised ability to form new blood vessels, and ability to restore endothelial integrity by vasculogenesis. In this regard, a previous experimental study demonstrates that neoangiogenesis by EPCs prevents apoptosis of hypertrophied myocardium, reduces progressive collagen deposition and scar formation, and improves ventricular function [24]. It might be suggested that patients with reduced EPCs and greater LV mass/BSA may have had a different equilibrium between CPCs and EPCs leading to a worse LV remodeling. Finally, the influence of a technical issue on the different behavior of CPCs and EPCs cannot be excluded. In fact it should be considered that EPCs are very rare in the circulation, and thus CPCs can be more accurately measured due to a higher number with respect to EPCs.

Once patients were divided according to the LV mass/BSA pre-established cutoff, we did not find any difference in relation to patients' ages. This finding is noteworthy, considering that in keeping with earlier studies [25–27], an inverse relationship between CPCs levels and age was evidenced in our study. The evidence suggests that progenitor cells are subject to age-associated changes, with an impairment of their function at older ages, and these changes may be involved in the dysregulation of endogenous cardiovascular repair mechanisms in the aging heart [28]. CPCs number proved to be significantly higher in women. This had already been observed in the past, and was mainly attributed to sex steroids [29–31]. We found, as previously suggested [32], the existence of a reduced number of CPCs in patients with ischemic heart disease. We cannot analyze in detail the role of therapy, even if a significant higher proportion of statin use was observed in patients without severely abnormal LVmass/BSA. This observation is in accordance with previous findings, concerning the role of statins in increasing the number of progenitor cells [33, 34]. Our results should be taken into account in the light of previous experimental results concerning the capability of bone marrow-derived cells to differentiate in vivo into cells expressing the properties of heart cells, possibly able to help in reconstituting the damaged myocardium. Moreover, it should be considered that previous studies [35, 36], indicate a role for bone marrow-derived progenitor cells in determining a favorable or unfavorable LV remodeling in patients with congestive HF. It is not possible to evaluate, on the basis of our results, the existence of a causal relationship between progenitor cells levels and ventricular remodeling. Nevertheless, it is a matter of fact that the behavior of ventricular remodeling parallels the behavior of progenitor cells, at least in the chronic HF patient population under study. If these results are confirmed by subsequent and more extensive investigations, the evaluation of progenitor cells, together with standard echocardiographic data, and possibly other non-invasive indicators of endothelial function (endothelium-dependent flow-mediated vasodilation (FMD)) could help in the initial evaluation of patients presenting with chronic heart failure, and might be useful in predicting the probability of response to heart failure therapy (e.g. cardiac re-synchronization therapy).

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Conflict of interest None.

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