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CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH ADVANCED HEART FAILURE AND EVOLUTION AFTER CARDIAC RESYNCHRONIZATION THERAPY

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CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN PATIENTS WITH ADVANCED HEART FAILURE AND EVOLUTION AFTER CARDIAC RESYNCHRONIZATION THERAPY

CÉLULAS PROGENITORAS ENDOTELIAIS CIRCULANTES EM DOENTES COM INSUFICIÊNCIA CARDÍACA AVANÇADA E EVOLUÇÃO APÓS TERAPÊUTICA DE RESSINCRONIZAÇÃO CARDÍACA

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ABSTRACT

Aims

Recent studies suggest that circulating endothelial progenitor cells (EPCs) may influence the response to cardiac resynchronization therapy (CRT). The aim of this study was to evaluate the effect of CRT on EPCs levels and to assess the impact of EPCs on long-term clinical outcomes.

Population and methods

Prospective study of 50 patients submitted to CRT. Two populations of circulating EPCs were quantified previously to CRT implantation: $CD34^+KDR^+$ and $CD133^+KDR^+$ cells. EPCs levels were reassessed 6 months after CRT. Endpoints during the long-term follow-up were all-cause mortality, heart transplantation and hospitalization for heart failure (HF) management.

Results

The proportion of non-responders to CRT was 42% and tended to be higher in patients with an ischemic vs non-ischemic etiology (64% vs 35%, p = 0.098). Patients with ischemic cardiomyopathy (ICM) showed significantly lower $CD34^+KDR^+$ EPCs levels when compared to non-ischemic dilated cardiomyopathy patients (DCM) (0.0010 ± 0.0007 vs 0.0030 ± 0.0024 cells/100 leukocytes, p = 0.032). There were no significant differences in baseline EPCs levels between survivors and non-survivors nor between patients who were rehospitalized for HF management during follow-up or not. At 6-month follow-up, circulating EPCs levels were significantly higher than baseline levels (0.0024 ± 0.0023 vs 0.0047 ± 0.0041 $CD34^+KDR^+$ cells/100 leukocytes, p = 0.010 and 0.0007 ± 0.0004 vs 0.0016 ± 0.0013 $CD133^+KDR^+$ cells/100 leukocytes, p = 0.007).

Conclusions

Patients with ICM showed significantly lower levels of circulating EPCs when compared to their counterparts. CRT seems to improve the pool of endogenously circulating EPCs and reduced baseline EPCs levels seem not influence long-term outcomes after CRT.

Keywords:

Endothelial Progenitor Cells; Cardiac Resynchronization Therapy; Heart Failure; Prognosis.

RESUMO

Introdução

Estudos recentes sugerem que as células progenitoras endoteliais (EPCs) circulantes podem participar na resposta à terapêutica de ressincronização cardíaca (TRC). O objetivo deste estudo foi avaliar o efeito da TRC nos níveis de EPCs circulantes e avaliar o impacto das EPCs no prognóstico a longo prazo.

População e métodos

Estudo prospetivo de 50 doentes submetidos a TRC. Antes da implantação, foram quantificadas 2 populações de EPCs circulantes por citometria de fluxo: células $CD34^+KDR^+$ e $CD133^+KDR^+$. Os níveis destas EPCs foram reavaliados 6 meses após TRC. Os *endpoints* durante o seguimento a longo prazo foram mortalidade por todas as causas, transplantação cardíaca e hospitalização por insuficiência cardíaca (IC).

Resultados

A proporção de não respondedores à TRC foi de 42%, tendendo a ser maior nos doentes com etiologia isquémica versus não isquémica (64% vs 35%, p = 0.098). Os doentes com miocardiopatia isquémica (MCI) apresentavam níveis significativamente mais baixos de EPCs $CD34^+KDR^+$ quando comparados aos doentes com miocardiopatia dilatada não-isquémica (MCD) (0.0010 ± 0.0007 vs 0.0030 ± 0.0024 células/100 leucócitos, p = 0.032). Não se verificaram diferenças significativas nos níveis basais de EPCs entre sobreviventes e não sobreviventes, nem entre doentes com ou sem necessidade de internamento para tratamento da IC durante o seguimento. Aos 6 meses de seguimento, os níveis de EPCs circulantes eram significativamente maiores do que os níveis basais (0.0024 ± 0.0023 vs 0.0047 ± 0.0041 $CD34^+KDR^+$/100 leucócitos, p = 0.010 e 0.0007 ± 0,0004 vs 0.0016 ± 0.0013 $CD133^+KDR^+$/100 leucócitos, p = 0.007).

Conclusões

Os doentes com MCI apresentam níveis basais de EPCs circulantes significativamente mais baixos que os seus homólogos. A TRC parece melhorar o *pool* endógeno de EPCs circulantes e níveis basais reduzidos de EPCs não parecem influenciar os *outcomes* a longo prazo após a TRC.

Palavras-chave:

Células Progenitoras Endoteliais; Terapia de Ressincronização Cardíaca; Insuficiência cardíaca; Prognóstico.

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LIST OF ABBREVIATIONS

ACE - angiotensin converting enzyme
ASA - acetylsalicylic acid
BB - β-adrenergic blockers
BNP - brain natriuretic peptide
CD - cluster of differentiation
CHF - congestive heart failure
CKD - chronic kidney disease
CRT - cardiac resynchronization therapy
DCM - non-ischemic dilated cardiomyopathy
EDTA - ethylenediaminetetraacetic acid
EPCs - endothelial progenitor cells
FITC - fluorescein isothiocyanate
HF - heart failure
HFrEF - heart failure with reduced ejection fraction
HR - heart rate
ICM - ischaemic cardiomyopathy
IL - interleukin
KDR - kinase insert domain receptor
LVEDV - left ventricular end-diastolic volume
LVEF - left ventricular ejection fraction
LVESV - left ventricular end-systolic volume
NOS - nitric oxide synthase
NYHA - New York Heart Association
PBS - phosphate buffered saline
PE - phycoerythrin
SDF-1 - stromal cell-derived factor 1
TNF - tumor necrosis factor
VEGF - vascular endothelial growth factor

INTRODUCTION

Advanced heart failure (HF) is associated with endothelial dysfunction[1–3] which negatively impacts cardiac function, heart failure progression and survival.[4] Circulating endothelial progenitor cells (EPCs) contribute to endothelial homeostasis and may serve as a circulating reservoir for endothelial repair in various pathological conditions.[5] Accumulating evidence shows that reduced circulating EPCs levels accurately reflect endothelial dysfunction.[6]

In patients with coronary artery disease, reduced EPCs levels have been identified as an independent predictor of future cardiovascular events.[7,8] However, in advanced HF, the association between circulating EPCs and the subsequent long-term clinical outcome remains undefined.

Cardiac resynchronization therapy (CRT) is a well-recognized and important treatment for patients with advanced HF.[9] However, some patients do not respond positively to CRT. Previous studies suggest that endothelial dysfunction may hamper response to CRT.[10,11] Moreover, previous work by our group suggest that circulating EPC levels may influence CRT response.[12] Nevertheless, no previous studies have specifically focused on the relation of circulating EPCs to subsequent long-term outcomes of advanced HF patients submitted to CRT nor about the effect of CRT on circulating EPCs levels.

The objectives of this study were to assess the potential value of circulating EPCs as a predictor of response to CRT and its association with long-term clinical outcomes. In addition, it was intended to study the impact of CRT on circulating EPCs pool.

POPULATION AND METHODS

Study Population

This is a prospective study of 50 patients with advanced HF undergoing cardiac resynchronization therapy (CRT) between 11/2009 and 10/2011 in a single centre. Demographic, clinical parameters (including New York Heart Association [NYHA] classification) and echocardiographic parameters of each patient were assessed before and 6 months after CRT. All patients were under stable, optimized medical therapy for CHF at the time of inclusion.

Inclusion criteria were a left ventricular ejection fraction (LVEF) ≤ 35%, QRS ≥ 120 ms with a left bundle branch block morphology and presence of sinus rhythm.

Exclusion criteria were: congenital heart disease, severe valvular disease, acute coronary syndrome, or percutaneous coronary intervention within the preceding 3 months, myocardial revascularization surgery or implantation of a previous cardiac pacing device, severe peripheral arterial occlusive disease, anemia (hemoglobin < 8.5 g/dL), renal insufficiency (creatinine > 2.0 mg/dL) or severe, noncontrolled, arterial hypertension (systolic blood pressure > 180 mmHg or diastolic > 110 mmHg), recent major bleeding requiring blood transfusion (< 6 months), concomitant inflammatory or infectious disease, autoimmune or neoplastic disease, trauma or surgery in the last month, cardiogenic shock, pregnancy, patients taking regular non-steroidal anti-inflammatory drugs or patients taking vasoactive amines or anticoagulants, comorbidities associated with a life expectancy of less than 1 year, and excessive alcohol consumption or illicit drugs abuse.

All study patients signed an informed consent, the study being accepted by the local ethics committee and in accordance with the criteria of the Declaration of Helsinki.

Echocardiographic Evaluation

Standard echocardiography was performed using Vivid 7 echocardiographs (GE Healthcare, Oslo, Norway) and a 1.7-3.4 MHz tissue harmonic transducer; appropriate software was used (EchoPAC, GE Healthcare). Left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV) and LVEF were calculated by the biplane Simpson´s equation in apical four-chamber and two-chamber views.

Long-term follow-up

Data on mortality, heart transplantation and hospitalization due to worsening heart failure were collected from reviewing hospital records at the closure of the study (April 2018). The echocardiogram performed 6-months after the implantation was used to assess response to CRT. Patients who demonstrated at least a 15% reduction in LVESV at the 6-month follow up were defined as responders to CRT.

Quantification of circulating EPCs by flow cytometry

Blood samples were collected to evaluate the analytical parameters (including brain natriuretic peptide [BNP]), just before the device implantation. In addition, venous blood samples, stored in ethylenediaminetetraacetic acid (EDTA) tubes, were also collected for quantification of circulating EPCs levels, and processed within 1 to 2 hours after collection. Hence, 150 µl of whole blood were incubated with 3 antihuman monoclonal antibody (mAB): 10 µl of APC-conjugated CD133 (Miltenyi Biotec Inc., Auburn, CA, USA), 10 µl of phycoerythrin (PE) conjugated KDR mAB (type 2 vascular endothelial growth factor receptor - VEGF-R2) (Sigma-Aldrich Co., St. Louis, MO, USA), 10 µl of fluorescein isothiocyanate (FITC) conjugated CD34 mAB (Becton Dickinson and Co.) for 30 min at 4°C, in the dark. For erythrocyte lysis FACS Lysing Solution (BD Biosciences) diluted in a ratio of 1:10 (vol/vol) with distilled water was used. Subsequently a wash with phosphate buffered saline (PBS) was performed. Further flow cytometric analysis was performed on all cases to evaluate for doublets, using a plot of FSC area versus FSC height. The data acquisition was performed in a high-performance flow cytometer, FACSCanto II (BD Biosciences). The Infinicyt 1.7 software (Cytognos, Salamanca, Spain) was used for the analysis. According to the standardized protocol, human circulating EPCs were identified by a minimal antigenic profile that includes at least one marker of immaturity (CD34 and/or CD133), plus at least one marker of endothelial commitment (KDR).

Because EPCs are extremely rare events in peripheral blood, in order to increase the sensitivity of the method and the accuracy of our work we increased the total number of acquired events to at least 1 million per sample.

Four different populations of angiogenic cells were quantified: $CD34^+$, $CD133^+$, $CD34^+KDR^+$ and $CD133^+KDR^+$. In the first 30 patients include in the study, these 4 populations were reassessed at 6-moth follow-up.

Statistical analysis

Statistical analyses were performed using SPSS software version 24 (IBM Corp., Armonk, NY, USA). Continuous variables were tested for normal distribution by Kolmogorov-Smirnov test and expressed as mean ± standard deviation or median ± interquartile range for parametric and nonparametric data, respectively. Categorical data are expressed as counts and percentages. For comparison of continuous data, we used unpaired Student t-test or nonparametric Mann–Whitney test for variables without a normal distribution. For the comparison of baseline and 6 months follow-up variables, the paired Student t-test or the Wilcoxon test was used, whichever appropriate. Categorical variables were compared with the chi-square test or with Fisher exact test as appropriate. Kaplan-Meier survival curves were used to evaluate the impact of EPCs levels on time-dependent clinical outcomes. Differences between pairs of survival curves were tested by the log-rank test.

The relationship between variables was calculated using Pearson's or Spearman's correlation coefficient, whichever appropriate. A two-tailed P value of < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Among the 50 patients with advanced HF, 11 patients (22%) had an ischemic and 39 a non-ischemic etiology. Mean age was 61.7 ± 10.5 years and most patients were male (64.0 ± 48.5%). The majority of patients were in NYHA class III (76.6%), with 10.6% in class II and 12.8% in ambulatory class IV before CRT. The global population had a left ventricular ejection fraction (LVEF) of 23.3 ± 6.8%, a heart rate (HR) of 70.2 ± 14.6 beats/minute and a QRS duration of 143.4 ± 29.0 ms.

Regarding the type of device implanted, the proportion of CRT-D versus CRT-P was 85.7/14.3%. Regarding the chronic medication, 72.1% of the patients were under angiotensin converting enzyme inhibitors (ACE inhibitors), 88.4% under beta adrenergic blockers (BB), 60.5% under spironolactone, 97.7% under furosemide, 34.9% under digoxin, 60.5% under statins, 34.9% under aspirin (ASA), and 14.0% under ivabradine. As expected, the proportion of patients treated with statins and ASA was significantly higher in the group of patients with ischemic cardiomyopathy (ICM).

Table 1 – Comparison baseline characteristics between ischemic and non-ischemic patients.

	Ischemic etiology (n = 11)	Non-ischemic etiology (n = 39)	P value
Age (years)[a]	61.5 ± 9.4	61.8 ± 10.9	0.920
Male gender (%)[a]	100.0	53.8	0.004
Years since diagnosis[a]	7.4 ± 5.3	5.77 ± 6.0	0.455
NYHA[a]	2.9 ± 0.3	3.1 ± 0.5	0.390
HR (beats/min)[a]	60.5 ± 7.4	72.8 ± 15.0	0.032
QRS (ms)[a]	130.0 ± 16.3	147.7 ± 31.1	0.093
Diabetes (%)	36.4	18.4	0.209
CKD (%)	10.0	19.4	0.497
Hypertension (%)	55.6	26.5	0.098
Hyperlipidemia (%)	80.0	40.0	0.026
Statin (%)	90.9	50.0	0.016
Acetylsalicylic acid (%)	72.7	21.9	0.002
ACE-inhibitor (%)	72.7	71.9	0.958
AT-1 blocker (%)	9.1	15.6	0.600
Beta-blocker (%)	90.9	87.5	0.768
Spironolactone (%)	45.5	65.6	0.248
Furosemide (%)	90.9	100.0	0.088
Ivabradine (%)	9.1	15.6	0.600
Digoxin (%)	36.4	34.4	0.908
CRT-D versus CRT-P (%)	100.0/0.0	81.3/18.8	0.308

[a]Mean ± standard deviation.

ACE = angiotensin-converting enzyme; CKD = chronic kidney disease; CRT-D = cardiac resynchronization therapy-defibrillator; CRT-P = cardiac resynchronization therapy-pacemaker; HR = heart rate; NYHA = New York Heart Association.

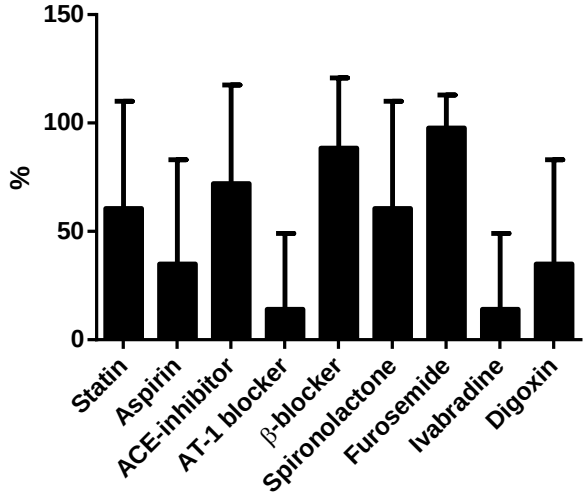


Figure 1 – Usual medication of the global study population.

Patients with ICM were more frequently male and had a higher proportion of cardiovascular risk factors (diabetes, hypertension and hyperlipidemia) than patients with non-ischemic cardiomyopathy (DCM) (Table 1). Moreover, the HR was significantly lower in ICM compared to DCM.

Table 2 – Comparison of pre-implantation echocardiographic parameters between ischemic and non-ischemic patients.

	Ischemic etiology (n = 11)	**Non-ischemic etiology (n = 39)**	**P value**
LVESV (mL)[a]	157.7 ± 35.0	200.1 ± 98.5	0.193
LVEDV (mL)[a]	218.3 ± 37.9	250.1 ± 106.2	0.363
LVEF (%)[a]	26.5 ± 6.3	22.3 ± 6.8	0.078
BNP (pg/mL)[a]	381.1 ± 330.5	550.0 ± 602.5	0.458

[a]Mean ± standard deviation.

BNP = brain natriuretic peptide; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume.

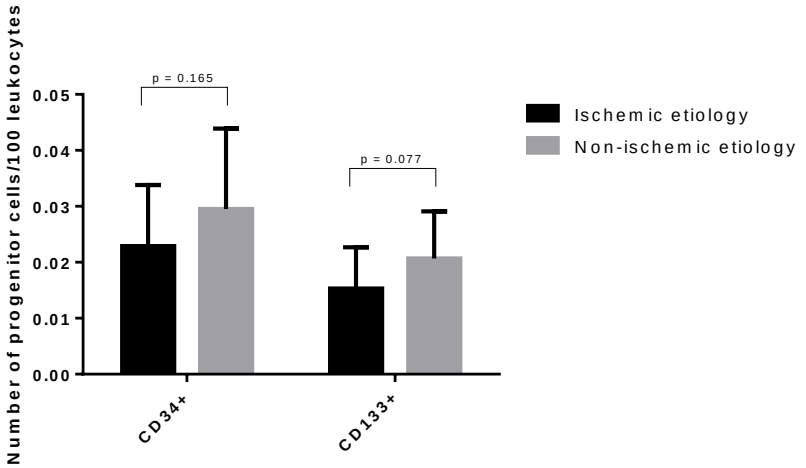
Patients with DCM tended to have a lower LVEF value when compared to patients with ICM (22.3 ± 6.8% versus 26.5 ± 6.3%, p = 0.078, respectively) (Table 2).

Circulating EPCs levels according to ischemic and non-ischemic etiology

There were no statistically significant differences in levels of circulating $CD34^+$, $CD133^+$ or $CD133^+KDR^+$ cells between ischemic and non-ischemic patients (Figure 2). However, the $CD133^+$ angiogenic cells tended to circulate in lower numbers in patients with ICM compared

to patients with an DCM (Figure 2A and 3). Levels of circulating $CD34^{+}KDR^{+}$ EPCs were significantly lower in patients with non-ischemic etiology (Figure 2B and 3).

(A)



(B)

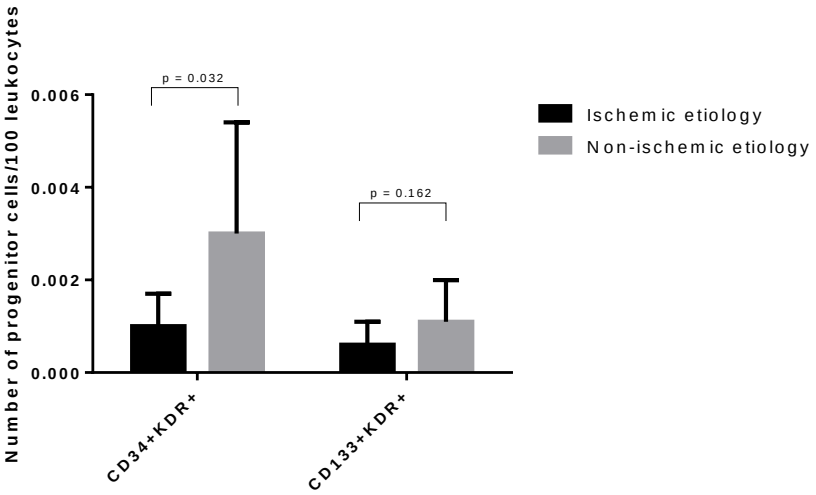
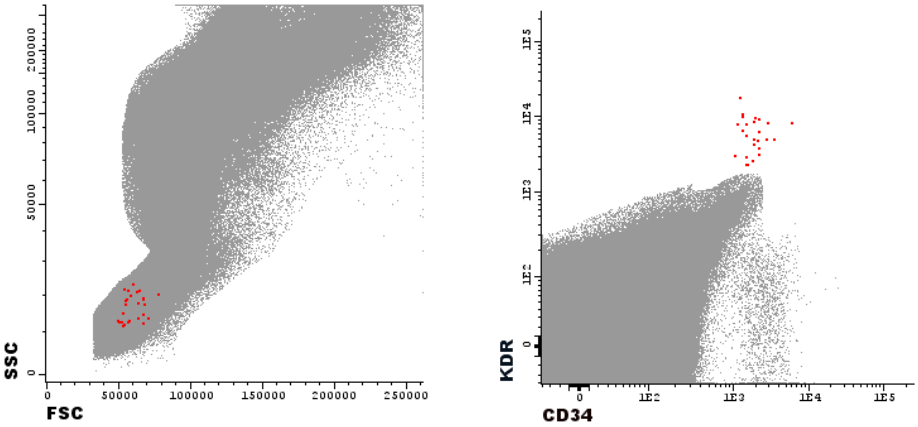


Figure 2 – EPCs levels according to ischemic or non-ischemic etiology. **(A)** Comparison of circulating levels of angiogenic $CD34^{+}$ and $CD133^{+}$ cells between ischemic and non-ischemic patients. **(B)** Comparison of $CD34^{+}KDR^{+}$ and $CD133^{+}KDR^{+}$ EPCs levels between ischemic and non-ischemic patients.

(A)



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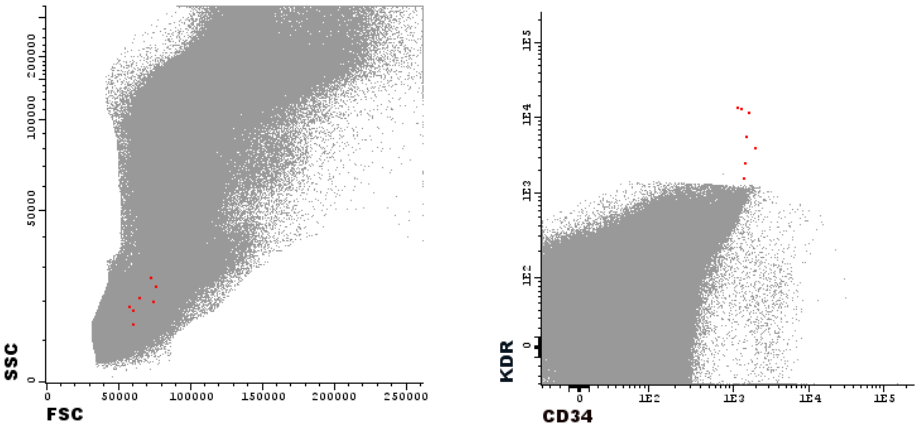

Figure 3 – Representative cytograms of EPCs quantification by flow cytometry in a patient with non-ischemic **(A)** and ischemic etiology of the cardiomyopathy **(B)**.

Long-term outcome after CRT

At 6-month follow-up, we verified a significant improvement in LVEF, with a significant decrease in LV volumes (Table 3). However, 42% of patients did not respond favourably to CRT.

We found no significant differences in baseline EPCs levels between responders and non-responders to CRT (0.0027 ± 0.0026 vs 0.0024 ± 0.0021 $CD34^{+}KDR^{+}$ cells, p = 0.669 and 0.0010 ± 0.0009 $CD133^{+}KDR^{+}$ cells, p = 0.858, respectively).

Table 3 – Evolution of echocardiographic parameters from baseline to 6-month follow-up.

	Before the device implantation (n = 50)	6-Month follow up (n = 50)	P value
LVEF (%)	23.7 ± 6.8	31.5 ± 11.0	<0.001
LVEDV (mL)	242.4 ± 95.7	217.6 ± 105.5	0.017
LVESV (mL)	189.0 ± 89.7	156.4 ± 96.5	0.004
BNP (pg/mL)[a]	498.9 ± 614.4	420.2 ± 497.8	0.337

The proportion of non-responders to CRT tended to be higher in patients with an ischemic etiology by comparison with non-ischemic patients (64% versus 35%, p = 0.098) (Table 4).

Table 4 – Comparison of clinical evolution between ischemic and non-ischemic patients.

	Ischemic etiology (n = 11)	Non-ischemic etiology (n = 39)	P value
Number of hospitalizations	1.8 ± 2.0	0.8 ± 1.3	0.052
Rehospitalization for HF (%)	63.6	38.5	0.137
Time Until First Release (months)	46.8 ± 40.1	53.1 ± 35.4	0.429
CV death (%)	36.4	35.9	0.977
Heart transplantation (%)	9.1	2.6	0.329
Responders (%)	36.4	64.7	0.098

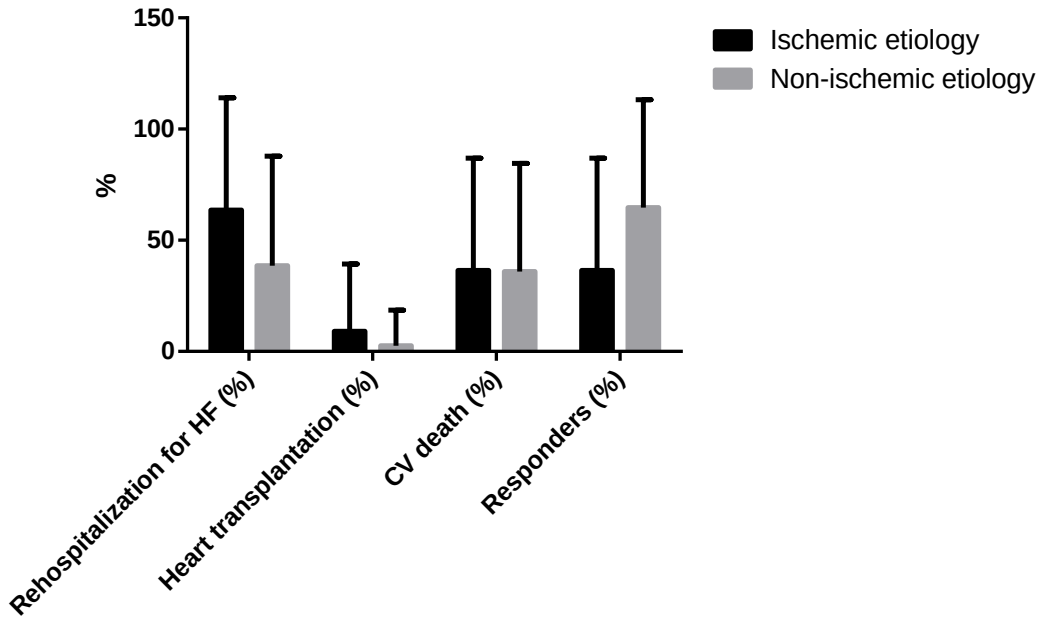

Figure 4 – Clinical outcomes after cardiac resynchronization therapy.

Regarding long-term clinical outcome (mean follow-up of 5.4 ± 2.3 years), 18 patients died: 5/29 (17%) in the responder group and 13/21 (61%) in non-responders (p = 0.019); 2 patients underwent heart transplantation (one responder and one non-responder) and 22 patients were re-hospitalized due to heart failure: 8/29 (28%) in responder group and 14/21 (67%) in non-responders to CRT (p = 0.039).

During follow-up, there were no statistically significant differences in mortality rate, or heart transplantation rate between ischemic and non-ischemic patients (Table 4). However, patients with ICM tended to be more often hospitalized due to HF than DCM patients (mean number of hospitalizations: 1.8 ± 2.0 vs 0.8 ± 1.3, p = 0.052, respectively and hospitalization rate: 63.6% vs 38.5%, p = 0.137, respectively (Table 4 and Figure 4).

There were no significant differences in baseline EPCs levels among patients who were alive and patients who died during long-term follow-up nor between patients who were rehospitalized for heart failure management or not (Figure 5). Additionally, there was no correlation between baseline EPCs levels and time to rehospitalization, number of rehosts, or time to mortality (Figure 6).

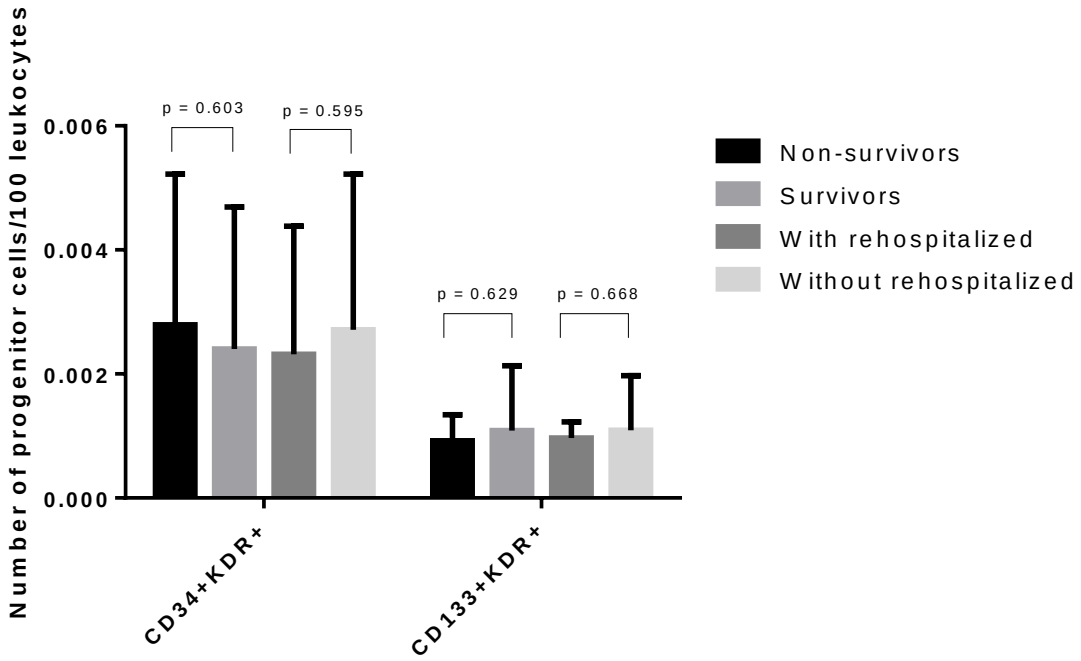

Figure 5 – Baseline EPCs levels among patients who were alive and patients who died during long-term follow-up and patients who were rehospitalized for heart failure management or not.

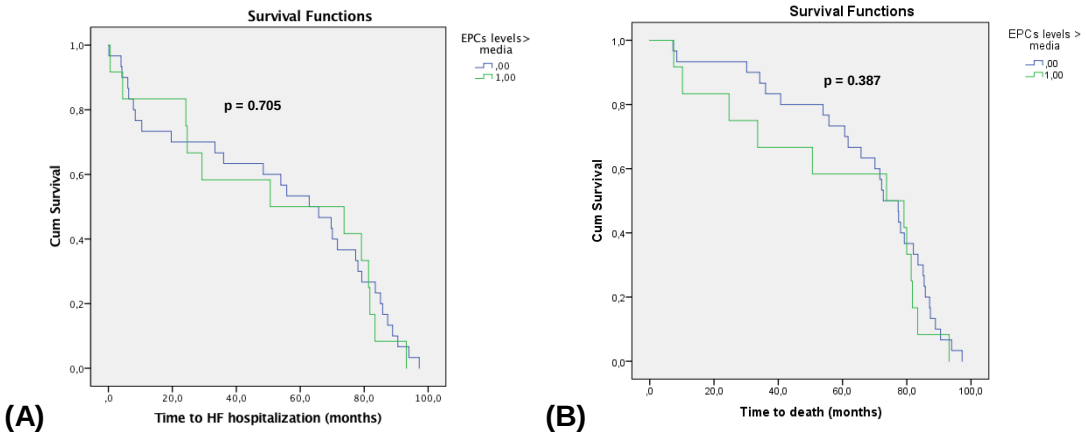

Figure 6 – Survival curve for time to first HF rehospitalization (months) **(A)** and for all-cause death (months) **(B)** according to baseline EPCs levels above media.

Evolution of EPC levels after CRT

Six-months after CRT patients presented significantly higher levels of both $CD34^{+}KDR^{+}$ and $CD133^{+}KDR^{+}$ EPCs than before the implantation (Table 5 and Figure 7). However, we did not find significant differences in the degree of increase in EPCs between responders and non-responders to CRT.

Table 5 – Comparison of EPCs levels before device implantation and after 6-month follow up.

	Before device implantation (n = 30)	6-month follow up (n = 30)	P value
CD34+ (%)	0.0275 ± 0.0135	0.0255 ± 0.0118	0.475
CD133+ (%)	0.0152 ± 0.0087	0.0180 ± 0.0080	0.280
CD34+KDR+	0.0024 ± 0.0023	0.0047 ± 0.0041	0.010
CD133+KDR+ (%)	0.0007 ± 0.0004	0.0016 ± 0.0013	0.007

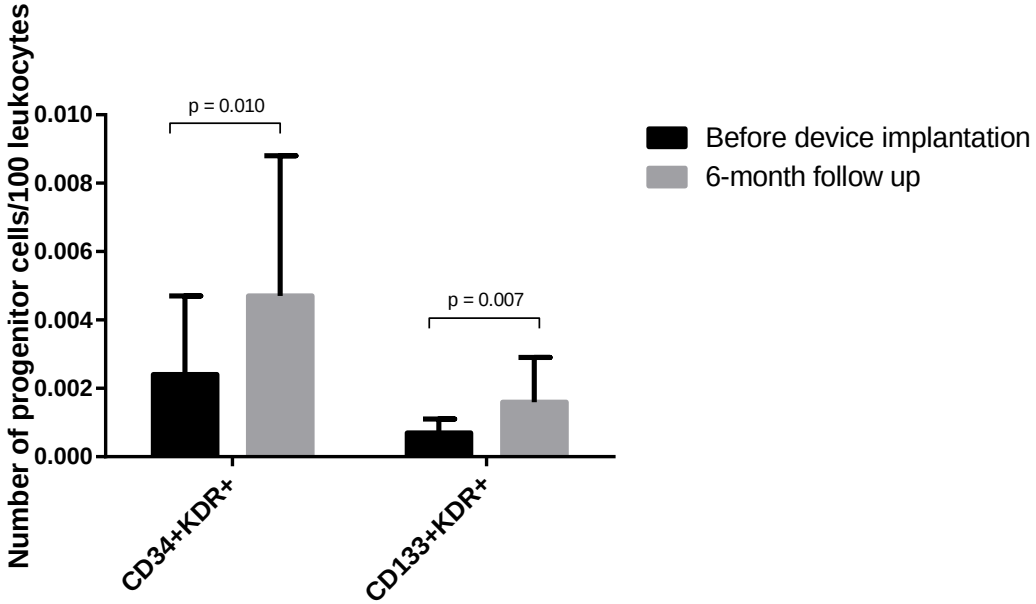

Figure 7 – EPCs levels before and six months after the implantation.

DISCUSSION AND CONCLUSIONS

DISCUSSION

To the best of our knowledge, this is the first study assessing the relation between circulating baseline EPCs and outcomes after CRT as well as the impact of CRT on circulating EPCs levels.

The main findings of the present work can be summarized as follows. First, the etiology of heart failure seems to influence EPCs levels, with lower number of circulating EPCs in the ischemic patients. Second, circulating levels of $CD34^{+}KDR^{+}$ and $CD133^{+}KDR^{+}$ cells significantly increase after CRT, independently of patient's response. Third, baseline EPCs numbers seem not to correlate with long-term outcome after CRT.

Heart failure with reduced ejection fraction (HFrEF) is a very common disease with an unacceptably poor prognosis. The prevalence of HF can be estimated at approximately 1–2% of the adult population in developed countries and the incidence approaches 5-10 per 1000 persons per year.[13]

Over the last two decades, CRT has revolutionized the treatment of selected patients who have HFrEF. CRT improves cardiac performance in appropriately selected patients and reduces morbidity and mortality.[14–16] Several studies have demonstrated the efficacy of CRT in counteracting ventricular remodelling through the recovery of synchronous muscle contractility.[17] However, the exact mechanisms leading to the long-term benefits of CRT are not yet fully understood and other mechanisms than left ventricular reverse remodelling are likely involved, explaining the discordance frequently observed between clinical and remodeling response to CRT and also between CRT response and long-term outcomes.[18,19]

End-stage HF is the final common pathway for several different pathologies, with ischemic aetiology being responsible for the vast majority of cases, in developed countries.[20]

Previous studies have suggested that patients with ischemic etiology have a lower probability of response to CRT than non-ischemic patients.[10,11,21] The reasons for their lack of response to CRT are not well understood. In ischemic etiology, LV desynchrony may be related to segmental wall motion abnormalities due to the presence of myocardial scars or perfusion defects that cannot be resynchronized. Here, we verify that patients with ICM express significantly lower levels of circulating EPCs, suggesting that this pauperization may justify why ICM patients typically benefit less from CRT. However, several studies conducted in recent years have found that the benefits of CRT appear to be similar in HF regardless of

the underlying cause.[22,23] Therefore, presently, the decision to indicate CRT is not influenced by the etiology of HF.

Endothelial dysfunction has been extensively reported in patients with HF.[24] Endothelial damage or ischemia leads to liberation of several mediators, such as VEGF, stromal cell-derived factor 1 (SDF-1) or nitric oxide synthase (NOS). This cascade activation seems to stimulate the proliferation of EPCs in bone marrow and their release to bloodstream.[25–27] Circulating EPCs adhere to the injured endothelium, playing a crucial role in vascular repair . During recent years, accumulating evidence revealed that circulating EPCs showed reduced numbers and functional impairment within several cardiovascular diseases.[28] Valgimigli *et al.* were the first to evaluate the role of circulating EPCs in HF patients. They showed decreasing EPC levels with more advanced stages of congestive heart failure (CHF) indicated by higher NYHA classes and elevated NT-proBNP levels.[29] Chiang *et al.* also showed that HF patients present lower EPCs counts than controls.[30] The reduction of circulating EPCs levels in advanced HF may be justified by diffuse and severe endothelial damage. However, conflicting results about the behaviour of circulating EPCs in advanced HF have been published. Theiss *et al.* found that circulating EPCs were lower in patients with ICM than DCM but still higher than healthy controls.[31] Heeschen *et al.* observed a functional impairment of bone marrow-derived EPCs leading to a reduced migratory capacity into the circulation of patients with ischemic HF compared to healthy controls.[32] However, findings from other investigators groups indicate that the etiology of HF does not differentially affect circulating EPCs.[29,33] In the present study, despite the greater use of statins (a stimulus for EPCs)[34,35] in patients with ischemic etiology, they showed significantly lower levels of circulating EPCs when compared to non-ischemic patients. This inferiority in circulating EPCs levels was observed for both the $CD34^{+}KDR^{+}$ cells and for the more immature $CD133^{+}KDR^{+}$ population. That difference could potentially explain why ICM patients typically benefit less from CRT and the worse prognosis generally associated with ischemic etiology compared to non-ischemic causes of HF.

Long-term outcome after CRT

Low circulating EPC levels are associated with adverse outcomes in patients with coronary artery disease.[36] Additionally, recent studies demonstrated that in patients with moderate to severe aortic stenosis a relatively low number of EPCs is associated with cardiac death at follow up.[37] However, regarding CHF, Michowitz *et al.* showed that higher levels of EPCs independently predicted all-cause mortality among patients with CHF.[33] In contrast, Koller *et al.* showed that EPCs defined as $CD34^{+}CD45^{dim}KDR^{+}$ cells were a strong and independent

inverse predictor of mortality in patients with CHF.[11] Similarly, Samman Tahhan *et al.* demonstrated that lower EPCs counts were strongly and independently predictive of mortality.[38] On other hand, another study found that $CD34^+KDR^+$ levels were not related with the risk of mortality, composite outcomes, or hospital admissions in patients with ambulatory left ventricular ejection fraction < 40%.[39] However, the potential impact of circulating EPCs on clinical outcomes after CRT had not yet been studied. In our study, baseline EPCs levels were not related with long-term outcomes in HF submitted to CRT. Moreover, we did not find any association between baseline EPCs levels and response to CRT.

Evolution of EPC levels after CRT

An important observation of our study is that numbers of both EPCs populations ($CD34^+KDR^+$ cells and $CD133^+KDR^+$ cells) significantly increase after CRT. We can conjecture that this increase in EPCs is a result of effective CRT which may translate in an improved capacity of endothelial repair mediated by EPCs. However, the significance of this finding remains to be determined.

In recent years the role of EPCs in cardiovascular disease as well as the interplay between inflammation and endothelial progenitor cell biology have been discussed. In patients at an increased cardiovascular risk (diabetes mellitus, systemic hypertension and hyperlipidemia) EPCs show a decreased proliferative capacity[40–42], and present reduced levels in peripheral circulation.[8,43] In patients with advanced CHF, the majority of studies indicate that circulating EPCs levels are profoundly decreased.[29]

HF is characterized by a chronic inflammatory status with elevated pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6. This inflammatory milieu can negatively impact on circulating EPCs.[44]

CRT has revolutionized the care of patients with HFrEF and previous studies have shown that it reduces the inflammatory milieu of CHF.[45,46] Theodorakis *et al.* showed that IL-6 and TNF-α were reduced after 3 months of biventricular pacing.[45] In the present study, circulating EPCs significantly increase after CRT. Therefore, we can speculate that this anti-inflammatory action of CRT can be translated into increase in circulating levels of $CD34^+KDR^+$ and $CD133^+$KDR EPCs. However, these findings need confirmation and possible mechanisms to explain this association need further investigation.

LIMITATIONS

This study had a relatively small sample size and future larger studies would be important to confirm that circulating EPCs do not influence long-term prognosis of HF patients submitted to CRT. Another limitation of the study is the absence of a healthy control group, which could help to understand whether or not the increase in EPCs levels after CRT means a return towards normal levels.

We were not able to explore other functional characteristics of EPCs what might provide further understanding about the role of CRT on EPCs response and its contribution to HF pathogenesis.

CONCLUSIONS

Together, the present study shows that patients with ICM present a pauperization in the EPCs pool and it suggests that CRT may improve circulating EPCs levels. Additionally, reduced baseline EPCs numbers seem not influence long-term outcomes after CRT. However, further studies are warranted to better understand the role of EPCs in advanced HF and its potential relation to the beneficial effects of CRT.

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