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ORIGINAL ARTICLE

Clinical Trials Study

Endothelial progenitor cells mobilization after maximal exercise according to heart failure severity

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Abstract

BACKGROUND

Vascular endothelial dysfunction is an underlying pathophysiological feature of chronic heart failure (CHF). Patients with CHF are characterized by impaired vasodilation and inflammation of the vascular endothelium. They also have low levels of endothelial progenitor cells (EPCs). EPCs are bone marrow derived cells involved in endothelium regeneration, homeostasis, and neovascularization. Exercise has been shown to improve vasodilation and stimulate the mobilization of EPCs in healthy people and patients with cardiovascular comorbidities. However, the effects of exercise on EPCs in different stages of CHF remain under investigation.

AIM

To evaluate the effect of a symptom-limited maximal cardiopulmonary exercise

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testing (CPET) on EPCs in CHF patients of different severity.

METHODS

Forty-nine consecutive patients (41 males) with stable CHF [mean age (years): 56 ± 10, ejection fraction (EF, %): 32 ± 8, peak oxygen uptake (VO_2, mL/kg/min): 18.1 ± 4.4] underwent a CPET on a cycle ergometer. Venous blood was sampled before and after CPET. Five circulating endothelial populations were quantified by flow cytometry: Three subgroups of EPCs [$CD34^+/CD45^-/CD133^+$, $CD34^+/CD45^-/CD133^+/VEGFR_2$ and $CD34^+/CD133^+$/vascular endothelial growth factor receptor 2 ($VEGFR_2$)] and two subgroups of circulating endothelial cells ($CD34^+/CD45^-/CD133^-$ and $CD34^+/CD45^-/CD133^-/VEGFR_2$). Patients were divided in two groups of severity according to the median value of peak VO_2 (18.0 mL/kg/min), predicted peak VO_2 (65.5%), ventilation/carbon dioxide output slope (32.5) and EF (reduced and mid-ranged EF). EPCs values are expressed as median (25th-75th percentiles) in cells/10^6 enucleated cells.

RESULTS

Patients with lower peak VO_2 increased the mobilization of $CD34^+/CD45^-/CD133^+$ [pre CPET: 60 (25-76) *vs* post CPET: 90 (70-103) cells/10^6 enucleated cells, $P < 0.001$], $CD34^+/CD45^-/CD133^+/VEGFR_2$ [pre CPET: 1 (1-4) *vs* post CPET: 5 (3-8) cells/10^6 enucleated cells, $P < 0.001$], $CD34^+/CD45^-/CD133^-$ [pre CPET: 186 (141-361) *vs* post CPET: 488 (247-658) cells/10^6 enucleated cells, $P < 0.001$] and $CD34^+/CD45^-/CD133^-/VEGFR_2$ [pre CPET: 2 (1-2) *vs* post CPET: 3 (2-5) cells/10^6 enucleated cells, $P < 0.001$], while patients with higher VO_2 increased the mobilization of $CD34^+/CD45^-/CD133^+$ [pre CPET: 42 (19-73) *vs* post CPET: 90 (39-118) cells/10^6 enucleated cells, $P < 0.001$], $CD34^+/CD45^-/CD133^+/VEGFR_2$ [pre CPET: 2 (1-3) *vs* post CPET: 6 (3-9) cells/10^6 enucleated cells, $P < 0.001$], $CD34^+/CD133^+/VEGFR_2$ [pre CPET: 10 (7-18) *vs* post CPET: 14 (10-19) cells/10^6 enucleated cells, $P < 0.01$], $CD34^+/CD45^-/CD133^-$ [pre CPET: 218 (158-247) *vs* post CPET: 311 (254-569) cells/10^6 enucleated cells, $P < 0.001$] and $CD34^+/CD45^-/CD133^-/VEGFR_2$ [pre CPET: 1 (1-2) *vs* post CPET: 4 (2-6) cells/10^6 enucleated cells, $P < 0.001$]. A similar increase in the mobilization of at least four out of five cellular populations was observed after maximal exercise within each severity group regarding predicted peak, ventilation/carbon dioxide output slope and EF as well ($P < 0.05$). However, there were no statistically significant differences in the mobilization of endothelial cellular populations between severity groups in each comparison ($P > 0.05$).

CONCLUSION

Our study has shown an increased EPCs and circulating endothelial cells mobilization after maximal exercise in CHF patients, but this increase was not associated with syndrome severity. Further investigation, however, is needed.

Key Words: Chronic heart failure; Endothelial progenitor cells; Circulating endothelial cells; Maximal exercise; Cardiopulmonary exercise testing; Severity

Core Tip: Vascular endothelial dysfunction is an underlying pathophysiological feature of chronic heart failure (CHF). Exercise has been proven to increase the mobilization of endothelial progenitor cells (EPCs), which are involved in vascular endothelial restoration and neo-vascularization, in healthy people and patients with co-morbidities. However, the effect of exercise on EPCs in patients with CHF of different severity remains unknown. In the present study, we compared the mobilization of EPCs in CHF patients of different severity, according to functional markers, after a symptom-limited cardiopulmonary exercise testing. No differences were found between severity groups, indicating thus the beneficial effect of exercise in these patients.

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INTRODUCTION

Chronic heart failure (CHF) is a multifactorial clinical syndrome with an incidence between 1% and 2% per year in developed countries in all age categories, while increasing to > 10% in the age category > 70 years[1]. Prognosis of patients with CHF is poor as the survival rates do not exceed 50% within 4 years and 26.7% within 10 years from the diagnosis[2,3].

A characteristic pathophysiological feature of CHF is vascular endothelial dysfunction[4] and microcirculation abnormalities associated with CHF severity[5]. Systemic inflammation caused by secretion of cytokines leads to disruption of the vascular endothelial barrier and causes acute endothelitis[6]. Vessels show impaired vasodilation due to increased degradation and reduced bioavailability of the nitric oxide (NO)[6,7].

Exercise has a beneficial impact in the function of the vascular endothelium. It has been shown that it suppresses the generation of free radicals and oxidative stress, increases the bioavailability of NO and induces vasodilation, thereby improving the aerobic capacity[8,9]. Endothelial progenitor cells (EPCs) have been proven to be involved in the shielding of vascular protection, the restoring of dysfunctional and injured endothelium, the promotion of angiogenesis and the regulation of vascular homeostasis[10,11]. The level of EPCs seem to predict the occurrence of cardiovascular events and death from cardiovascular causes and may help to identify patients at increased cardiovascular risk[12]. Low counts of EPCs have been shown to be strongly and independently predictive of mortality in patients with cardiovascular comorbidities[13].

Regular aerobic exercise induces the mobilization of EPCs from the bone marrow, not only in the healthy population but also in populations with comorbidities and increased risk factors[14,15]. However, the effect of maximal exercise on EPCs in patients with CHF, and especially in patients of different severity, remains under investigation.

We hypothesized that maximal exercise has a beneficial effect on vascular endothelial function in patients with CHF irrespectively of their severity. The aim of the study was to assess, quantify and compare the acute mobilization of EPCs after maximal exercise in patients with CHF of both lower and higher severity.

MATERIALS AND METHODS

Study design

This interventional clinical study was conducted in accordance with the Declaration of Helsinki and approved by the Administration Board and the Ethics Committee of "Evaggelismos General Hospital" in Athens, Greece (Approval No. 117/3-7-2017). All of the patients signed an informed consent form in order to participate in the study. This is a post-hoc analysis study of a previous conducted research study published recently aiming to assess EPCs mobilization after exercise in patients with CHF[16]. Patients were referred for assessment to the "Clinical Ergospirometry, Exercise and Rehabilitation Laboratory" of "Evaggelismos General Hospital" by heart failure outpatient clinics of Athens. The diagnosis of CHF was based on personal history forms, clinical evaluation and laboratory testing of every patient.

Patients

The population of the study consisted of 49 consecutive patients with stable CHF and a reduced or mid-ranged ejection fraction (EF) who underwent a single session of symptom limited maximal cardiopulmonary exercise testing (CPET) on an electromagnetically braked cycle ergometer (Ergoline 800; SensorMedics Corporation, Anaheim, CA, United States). Inclusion criteria were stable CHF at maximum tolerated medication and EF ≤ 49%. Exclusion criteria were severe valvulopathy, uncontrolled arterial hypertension, severe chronic obstructive pulmonary disease, severe peripheral angiopathy, neuromuscular diseases and contraindications for

maximum cardiopulmonary stress testing[17].

Patients were divided in groups according to syndrome severity. CPET indices [peak oxygen uptake (VO_2), predicted peak VO_2 and ventilation (VE)/carbon dioxide output (VCO_2)] were used in order to divide these patients in two groups for each parameter. Cut off values (medians) were set for each of these parameters; a value of 18.0 mL/kg/min was set for peak VO_2, a value of 65.5% for predicted peak VO_2 and a value of 32.5 for VE/VCO_2. The demographic and exercise characteristics between severity groups divided by peak VO_2 are shown in Table 1 and for the other parameters in Supplementary Tables 1-3).

Patients were also divided in two groups according to their EF. The first group consisted of patients with CHF with a reduced EF (< 40%), while the second group included patients with CHF with a mid-ranged EF (40%-49%).

CPET

Patient performed a ramp-incremental exercise test, using the Hansen *et al*[18] equation for individual work rate increments, so as to aim for a test of 8-12 min duration. The nose of the patients was clamped, and they breathed through a special mask with a low resistance valve and a known gas mixture. Breathing parameters such as VO_2, VCO_2 and VE were measured in each breath by the software, and their values were recorded at the monitor of the computer system (Vmax 229, Sensor Medics). The gas exchanges of each patient were also recorded in order to calculate more specific values such as resting VO_2, VO_2 at peak exercise (peak VO_2), predicted VO_2 at peak exercise (predicted peak VO_2) and VE/VCO_2 slope.

All of the measurements were usually recorded in four time points; the first was for 2 min at rest (baseline values), the second for 2 min of unloaded pedaling before the beginning of the exercise, the third during exercise and the last for 5 min during the recovery point. A 12-lead electrocardiogram system was also attached on the patient's body in order to monitor the heart rate and the heart rhythm, a pulse oxymeter on the patient's finger measured the saturation and blood pressure was measured very 2 min. The end point of the session was due to electrocardiogram abnormal rhythm at the monitor, dyspnea or leg fatigue of the patient.

The peak values for VO_2, VCO_2 and VE were calculated as the average of measurements made during the 20-s period before the end of exercise[19]. Peak work rate was defined as the highest work rate reached and maintained at a pedaling frequency of no less than 65 revolutions per min. The ventilatory response to exercise was calculated as the slope by linear regression of VE *vs* VCO_2 from the beginning of exercise to anaerobic threshold, where the relationship was linear[19].

Flow cytometry analyses

For evaluation of EPCs, blood samples were drawn from a peripheral vein of each patient, once before the CPET at rest and once just after the CPET. Venous blood was collected in ethylenediaminetetraacetic acid tubes. Blood samples were taken to the immunology laboratory within the first hour after the collection where they were measured with the use of flow cytometry. The protocol that we implemented was the Duda *et al*[20] protocol, where four types of monoclonal antibodies were used; CD45, CD34, CD133 and vascular endothelial growth factor receptor 2 ($VEGFR_2$, CD309). In the meantime, five different cellular populations, three subgroups of EPCs and two subgroups of circulating endothelial cells (CECs) were defined; these were $CD34^+/CD45^-/CD133^+$, $CD34^+/CD45^-/CD133^+/VEGFR_2$, $CD34^+/CD133^+/VEGFR_2$ (EPCs subgroups), $CD34^+/CD45^-/CD133^-$ and $CD34^+/CD45^-/CD133^-/VEGFR_2$ (CECs subgroups).

Four-color flow cytometry was performed in the Flow Cytometry Core Laboratory with BD FACSCantoII (Becton-Dickinson, Franklin Lakes, NJ, United States) flow cytometer. Each analysis on the flow cytometer included 1×10^6 events. The number of EPCs was expressed as absolute number of cells/10^6 enucleated cells (Figure 1).

Statistical analyses

Patients were divided according to CHF severity based on CPET assessment, and results are presented according to severity groups. Descriptive characteristics are expressed as mean ± standard deviation while values of cellular populations belong to non-normal distribution, and they are expressed in median (25^{th}-75^{th} percentiles). All categorical variables are presented as absolute and percentage values. Normality of distribution was checked with the Shapiro-Wilk test. We used the Spearman's correlation coefficient to assess the direction and the magnitude of the association between the absolute and percentage differences of each endothelial cellular

Table 1 Demographic characteristics and maximal cardiopulmonary exercise testing indices of patients with chronic heart failure of different severity based on peak oxygen uptake

Demographic characteristics	Group 1	Group 2
Patients, *n*	25	24
Gender, males/females	21/4	20/4
Age in yr[1]	57 ± 10	55 ± 9
Height in cm[1]	174 ± 11	175 ± 9
Weight in kg[1]	92 ± 25	87 ± 21
NYHA stage, class II/III	16/9	18/6
EF, %[1]	32 ± 9	32 ± 8
Type of CHF		
Dilated cardiomyopathy, *n* (%)	7 (28)	5 (21)
Ischemic, *n* (%)	14 (56)	15 (63)
Other, *i.e.* valvulopathy, *etc.*, *n* (%)	4 (16)	4 (17)
Medication		
Diuretics, *n* (%)	19 (76)	13 (54)
ACE inhibitors, *n* (%)	11 (44)	13 (54)
ARBs, *n* (%)	5 (20)	2 (8)
β-Blockers, *n* (%)	25 (100)	23 (96)
Aldosterone antagonists, *n* (%)	19 (76)	18 (75)
Cardiopulmonary exercise testing parameters		
Peak VO_2 in mL/kg/min[1]	14.5 ± 2.5	21.8 ± 2.4[a]
Predicted peak VO_2, %[1]	52 ± 13	74 ± 11[a]
VE/VCO2 slope[1]	34 ± 5	33 ± 4
Peak WR in watts[1]	75 ± 34	118 ± 34[a]

Group 1: Peak VO_2 < 18.0 mL/kg/min, Group 2: Peak VO_2 ≥ 18.0 mL/kg/min.

[1]Values are expressed as mean ± SD; Difference between the 2 severity groups for demographic characteristics and cardiopulmonary exercise testing parameters ([a]P < 0.05). ACE: Angiotensin-converting-enzyme; ARB: Angiotensin II receptor blockers; CPET: Cardiopulmonary exercise testing; CHF: Chronic heart failure; EF: Ejection fraction; NYHA: New York Heart Association; VCO_2: Carbon dioxide output; VO_2: Oxygen uptake; WR: Work rate.

population and the values of CPET parameters and EF. Unpaired two sample Student's *t* test analyzed differences in demographics and CPET parameters between severity groups. Wilcoxon signed-rank test for non-parametric data analyzed differences between cellular populations within severity groups. Differences between severity groups were assessed with factorial analysis of variance (ANOVA) 2 × 2 (time × group). Dependent variables were transformed with the natural logarithm when deviating from normality prior to entering the ANOVA models. All tests were two-tailed, and level of observed statistical significance was adjusted to 0.05. No adjustment for multiple comparisons was performed as ANOVA analyses assessed independent outcomes. Statistical analyses were performed with IBM SPSS 25 Statistics software (Armonk, NY, United States).

RESULTS

Both severity groups, which were divided according to the median value of peak VO_2, increased the mobilization of their endothelial cellular populations after a symptom-limited CPET (Table 2). In group 1 (peak VO_2 < 18.0 mL/kg/min), all endothelial cellular populations increased except for the $CD34^+/CD133^+/VEGFR_2$ EPCs population, while in group 2 (peak VO_2 ≥ 18.0 mL/kg/min) all endothelial cellular populations increased (Table 2). No differences in the mobilization of endothelial

Table 2 Acute mobilization of endothelial cellular populations after a symptom-limited cardiopulmonary exercise testing of two different severity groups according to the median value of peak oxygen uptake

Endothelial cellular populations	Group 1 of n = 25, peak VO_2 < 18.0 mL/kg/min		Group 2 of n = 24, peak VO_2 ≥ 18.0 mL/kg/min		P value between groups
	Before CPET	After CPET	Before CPET	After CPET	
$CD34^+/CD45^-/CD133^+$	60 (25-76)	90 (70-103)[e]	42 (19-73)	90 (39-118)[e]	0.329
$CD34^+/CD45^-/CD133^+/VEGFR_2$	1 (1-4)	5 (3-8)[e]	2 (1-3)	6 (3-9)[e]	0.075
$CD34^+/CD133^+/VEGFR_2$	13 (9-17)	13 (9-26)	10 (7-18)	14 (10-19)[b]	0.257
$CD34^+/CD45^-/CD133^-$	186 (141-361)	488 (247-658)[e]	218 (158-247)	311 (254-569)[e]	0.101
$CD34^+/CD45^-/CD133^-/VEGFR_2$	2 (1-2)	3 (2-5)[e]	1 (1-2)	4 (2-6)[e]	0.471

Difference within each severity group.
[b]P < 0.01.
[e]P < 0.001. CPET: Cardiopulmonary exercise testing; VO_2: Oxygen uptake.

cellular populations between the two severity groups were observed (Table 2 and Supplementary Figure 1). Figure 2 shows the difference between the two groups.

Regarding severity groups divided according to the median value of predicted peak VO_2, they both increased the mobilization of their endothelial cellular populations after maximal CPET (Supplementary Table 4). In group 1 (predicted peak VO_2 < 65.5%), all endothelial cellular populations increased, while in group 2 (predicted peak VO_2 ≥ 65.5%) all endothelial cellular populations increased except for the $CD34^+/CD133^+/VEGFR_2$ EPCs population (Supplementary Table 4). No differences in the mobilization of endothelial cellular populations between the two severity groups were observed (Supplementary Table 4).

As far as severity groups divided according to the median value of VE/VCO_2 slope are concerned, they both increased the mobilization of their endothelial cellular populations after maximal CPET (Table 3). In group 1 (VE/VCO_2 < 32.5), all endothelial cellular populations increased except for the $CD34^+/CD133^+/VEGFR_2$ EPCs population, while in group 2 (VE/VCO_2 ≥ 32.5) all endothelial cellular populations increased (Table 3). No differences in the mobilization of endothelial cellular populations between the two severity groups were observed (Table 3).

Finally, for severity groups divided according to their EF, they both increased the mobilization of their endothelial cellular populations after maximal CPET (Table 4). In group 1 (EF < 40%), all endothelial cellular populations increased, while in group 2 (EF ≥ 40%) all endothelial cellular populations increased except for the $CD34^+/CD133^+/VEGFR_2$ EPCs population (Table 4). No differences in the mobilization of endothelial cellular populations between the two severity groups were observed (Table 4).

A positive correlation between percentage difference in $CD34^+/CD45^-/CD133^-/VEGFR_2$ population and peak VO_2 was observed (r = 0.341, P = 0.017), while the numeric difference in the same population tended also to correlate positively (r = 0.252, P = 0.081, Supplementary Table 5). We defined new groups of patients according to the median value of the percentage increase of each endothelial cellular population's mobilization. It was revealed that demographics and CPET indices did not differ between the two groups for all endothelial cellular populations except for $CD34^+/CD45^-/CD133^-/VEGFR_2$ EPCs. Patients with greater increase of this latter EPC population after exercise were younger, had higher peak VO_2 and work rate peak and lower VE/VCO_2 slope (Supplementary Table 6).

DISCUSSION

Our present study demonstrated that a symptom-limited maximal CPET exercise stimulates the mobilization of EPCs and CECs in patients with CHF. However, the results of our study did not show any clear association of EPCs and CECs mobilization and CHF severity.

Attenuated endothelial function has been previously associated with decreased EPCs[21], and EPCs have been linked to the repair mechanism of endothelial damage[10,11].

Table 3 Acute mobilization of endothelial cellular populations after a symptom-limited cardiopulmonary exercise testing of different severity groups according to the median value of ventilation/carbon dioxide output slope

Endothelial cellular populations	Group 1 of *n* = 27, VE/VCO_2 slope < 32.5		Group 2 of *n* = 22, VE/VCO_2 slope ≥ 32.5		*P* value between groups
	Before CPET	After CPET	Before CPET	After CPET	
$CD34^+/CD45^-/CD133^+$	62 (41-81)	95 (81-118)[e]	31 (18-66)	70 (33-99)[e]	0.711
$CD34^+/CD45^-/CD133^+/VEGFR_2$	1 (1-3)	5 (3-8)[e]	2 (1-4)	5 (3-8)[e]	0.311
$CD34^+/CD133^+/VEGFR_2$	10 (7-16)	13 (10-18)	12 (8-18)	16 (9-29)[b]	0.134
$CD34^+/CD45^-/CD133^-$	222 (147-287)	419 (267-576)[e]	198 (152-376)	382 (249-794)[e]	0.540
$CD34^+/CD45^-/CD133^-/VEGFR_2$	1 (1-2)	3 (2-5)[e]	1 (1-2)	4 (3-6)[e]	0.464

Differences within each severity group.
[b]*P* < 0.01.
[e]*P* < 0.001. CPET: Cardiopulmonary exercise testing.

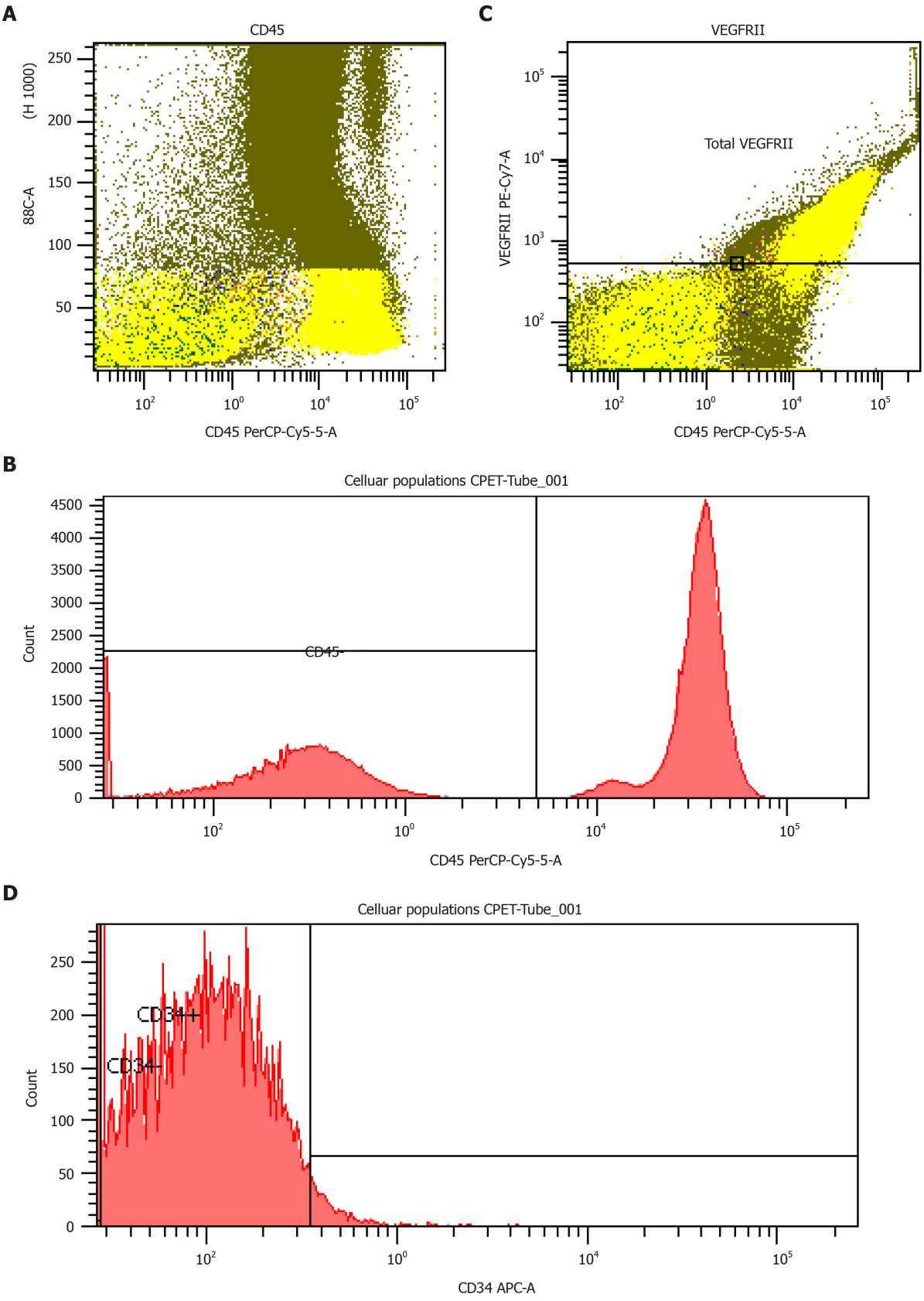
Table 4 Acute mobilization of endothelial cellular populations after a symptom-limited cardiopulmonary exercise testing of two different severity groups according to reduced or mid-ranged ejection fraction

Endothelial cellular populations	Group 1 of *n* = 37, EF < 40%		Group 2 of *n* = 12, EF ≥ 40%		*P* value between groups
	Before CPET	After CPET	Before CPET	After CPET	
$CD34^+/CD45^-/CD133^+$	42 (22-75)	90 (37-106)[e]	63 (40-76)	90 (65-103)[b]	0.888
$CD34^+/CD45^-/CD133^+/VEGFR_2$	2 (1-3)	5 (3-8)[e]	2 (1-4)	8(4-8)[b]	0.507
$CD34^+/CD133^+/VEGFR_2$	11 (7-17)	14 (10-23)[b]	15 (9-20)	13 (9-22)	0.473
$CD34^+/CD45^-/CD133^-$	200 (152-279)	427 (260-626)[e]	227 (135-372)	336 (214-624)[b]	0.702
$CD34^+/CD45^-/CD133^-/VEGFR_2$	1 (1-2)	3 (2-6)[e]	1 (1-2)	3 (2-5)[b]	0.828

Differences within each severity group.
[b]*P* < 0.01.
[e]*P* < 0.001. CPET: Cardiopulmonary exercise testing; EF: Ejection fraction.

To our knowledge only one single study has been conducted so far investigating the acute effect of maximal exercise on vascular endothelial function in patients with CHF and most specifically on the EPC populations[22]. This was the study of Van Craenenbroeck *et al*[22], who has investigated the effect of a single exercise bout in reversing endothelial dysfunction in CHF patients. Although Van Craenenbroeck *et al*[22] have shown a significant improvement in circulating angiogenic cell migratory capacity after exercise, they did not notice a significant increase in EPCs. In the present study, we extend previous findings showing that there is a significant EPCs mobilization after a symptom-limited maximal CPET in patients with CHF, but there was no significant difference between CHF severity groups. An explanation of these differences between the two studies may be the fact that we used different inclusion criteria and parameters for patient severity. In contrast with Van Craenenbroeck *et al*[22], who divided patients according to N-terminal pro-brain natriuretic peptide levels, in our study, we divided patients according to strong prognostic indicators such as peak VO_2, predicted peak VO_2, VE/VCO_2 slope and EF. Another possible explanation of the differences might be the methodology used in our study for the EPC quantification. In our study, we have used a more analytic EPC quantification with five endothelial populations defined with the use of four monoclonal antibodies, whereas Van Craenenbroeck *et al*[22] used two populations of endothelial cells for their determination (defined as $CD34^+/KDR^+/CD3^-$ and $CD34^+/CD3^-$ progenitor cells).

In a previous study from our institute, Stefanou *et al*[23] quantified three populations of EPCs in critically ill patients with sepsis including $CD34^+/CD45^-/CD133^+$, $CD34^+/CD45^-/CD133^+/VEGFR_2$ and $CD34^+/CD45^-/VEGFR_2$. In that study, neuromuscular electrical stimulation, considered as an alternative method of exercise, was applied to these patients showing that it could stimulate the mobilization of EPCs in all of the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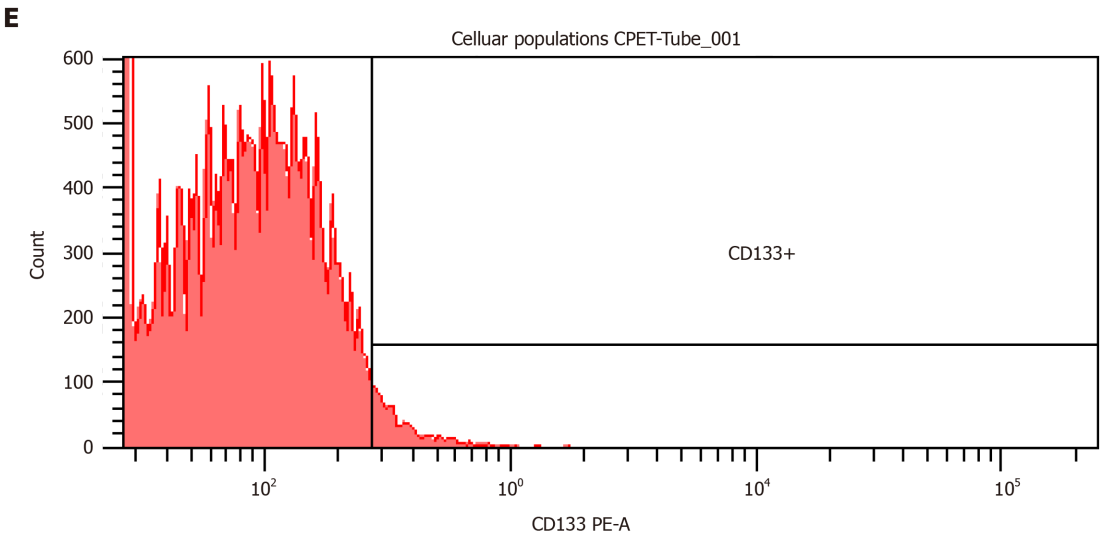

Figure 1 Boolean analysis. A and B: Flow cytometry analysis for the identification of cellular populations using monoclonal antibodies CD45; C: $VEGFR_2$; D: CD34; E: CD133. In all samples, the CD34 expression was weak. CPET: Cardiopulmonary exercise testing; $VEGFR_2$: Vascular endothelial growth factor receptor 2.

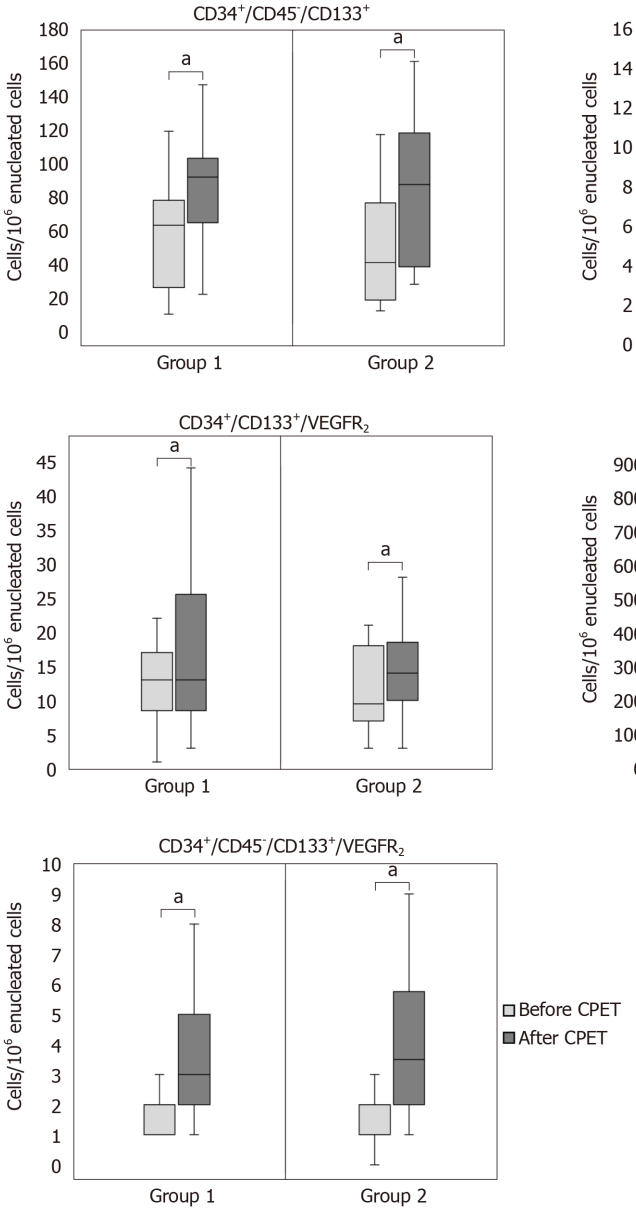

Figure 2 Boxplots representing the acute mobilization of each endothelial cellular population before and after a symptom limited maximal cardiopulmonary exercise testing between two severity groups according to the median value of peak oxygen uptake. Group 1: Peak oxygen uptake (VO_2) < 18.0 mL/kg/min; Group 2: Peak VO_2 ≥ 18.0 mL/kg/min). $^aP < 0.05$ indicates statistically significantly increase.

cellular populations mentioned. The findings of the Stefanou *et al*[23] study is in agreement with our findings as we also observed a mobilization of EPCs in all cellular populations after a single session of exercise training.

The novel insight of our study, in comparison with previous studies, is the assessment of the acute mobilization of EPCs after maximal exercise according to CHF severity by using strong prognostic CPET parameters such as peak VO_2 and VE/VCO_2 slope. An interesting finding from our study was that those patients who had a greater increase in a single EPCs population (*i.e.* $CD34^+/CD45^-/CD133^-/VEGFR_2$ cells population) were younger and had better CPET performance. The underlying mechanism of this finding cannot be discerned from the present study; secreting cytokines such as VEGF might interact more with this particular EPC population, and its secretion might be related with the degree of endothelium damage and the exercise stimuli. However, further study is needed to investigate this finding.

The present study has also introduced a more analytic methodology to quantify and define EPCs and CECs compared to previous studies. EPCs and CECs are being used as an index of the endothelium restoration potential and to reflect vascular endothelial function[10]. In our study, a large number of endothelial cellular populations was defined and quantified: Three groups of EPCs ($CD34^+/CD45^-/CD133^+$, $CD34^+/CD45^-/CD133^+/VEGFR_2$ and $CD34^+/CD133^+/VEGFR_2$) and two groups of CECs ($CD34^+/CD45^-/CD133^-$ and $CD34^+/CD45^-/CD133^-/VEGFR_2$) broadened our knowledge of the phenotype of endothelial cellular populations. Monoclonal antibodies such as CD45, CD34, CD133 and $VEGFR_2$ (CD309) are the most widely used for the definition of EPC and CEC phenotypes[20,24,25]. Other monoclonal antibodies such as CD146, CD105 and CD144 have been previously used, however, without providing more information or specialization in EPC or CEC phenotypes[24,26].

CHF is characterized by increased inflammatory status, endothelial dysfunction and impaired microcirculation, which are crucially involved in development and progression of the disease[27]. Impaired endothelium dependent vasodilatation, in addition to impaired cardiac function, is a main determinant of exercise intolerance in patients with CHF, limiting physical exercise capacity and deteriorating peak aerobic capacity[6,7,27]. Exercise has been reported to increase blood flow and shear stress, therefore increasing endothelial NOS activity and NO production and reducing inflammation[28]. Patients with CHF usually have a different level of deterioration of their vascular endothelium. However, through the present study, exercise was shown to enhance the acute mobilization of EPCs and CECs in these patients in a similar beneficial way, irrespectively of their severity. Targeting endothelial dysfunction could be a breakthrough therapy as endothelial function is recognized as a crucial component underlying HF.

Regarding the potential mechanisms of the mobilization of EPCs, shear stress could be suggested as a triggering factor for their release after a symptom limited maximal CPET. Shear stress seems to upregulate the activity of endothelial NO synthase and increase the production of NO[29,30]. Moreover, the activation of ion, cation and stretch sensitive channels and a transient increase in intracellular Ca^{2+} have been observed in endothelial cells immediately after exposure to shear stress[29,30]. All these endothelial functions contribute to the amplified number and activity of circulating EPCs, and they could play a role in signaling to the cell that it is under shear and eliciting a response[29,30].

Another possible mechanism that may be suggested is the ischemic/hypoxic stimulus. Ischemic/hypoxic stimulus has been shown to increase EPCs count in short-term studies enrolling patients with cardiovascular disease[31] and in patients with peripheral arterial occlusive disease[32]. Exercise has the potential to induce hypoxic stimuli, as suggested by alterations in microcirculation indices during exercise sessions in healthy populations and patients with cardiovascular comorbidities[33,34]. These mechanisms may relate to up-regulation of transcriptional factors, such as matrix metalloproteinases, stromal cell-derived factor 1 and vascular endothelial growth factor, which mediate processes to promote proliferative and migratory capacities of circulating EPCs[22,32,34].

Our study had certain limitations. This was a post-hoc analysis study not designed to compare CHF groups, making our analysis underpowered for group comparison. However, this is the larger sample size tested and analyzed to date and provides significant results according to CHF severity. Our results cannot be generalized to all CHF populations. We excluded patients with unstable and/or decompensated heart failure due to the inability to perform maximal CPET ,and for this reason our findings cannot be applied in such cohort of patients.

On the other hand, our study broadens horizons for future fields of research. The function and role of each cellular population in the vascular endothelium, the

relationship between cellular populations, local and systemic neurohumoral factors and cytokines or other vascular endothelium factors and exercise modalities (type, intensity, duration, volume) merits further investigation. Furthermore, other non-invasive methodologies reflecting endothelium function and microcirculation such as flow mediated dilation and near-infrared spectroscopy should be tested and investigated in relationship to EPC measurements to provide potential indirect evaluation of bone marrow response.

CONCLUSION

In conclusion, a single symptom-limited maximal CPET induces a significant mobilization of EPCs and CECs in CHF patients, but there was no significant association with disease severity.

ARTICLE HIGHLIGHTS

Research background

Vascular endothelial dysfunction is an underlying pathophysiological feature of chronic heart failure (CHF). Patients with CHF are characterized by impaired vasodilation and inflammation of the vascular endothelium. They also have low levels of endothelial progenitor cells (EPCs). EPCs have been used as an index of the endothelium restoration potential, therefore reflecting the vascular endothelial function. Exercise has a beneficial impact in the function of the vascular endothelium and EPCs.

Research motivation

Despite the proven beneficial effect of exercise training in patients with cardiovascular comorbidities, the effect of maximal exercise on EPCs in patients with CHF, and especially in patients of different severity, remains under investigation.

Research objectives

This study was conducted to assess, quantify and compare the acute mobilization of EPCs after maximal exercise in patients with CHF of both lower and higher severity.

Research methods

Forty-nine consecutive patients with stable CHF underwent a cardiopulmonary exercise testing (CPET) on a cycle ergometer. Venous blood was sampled before and after CPET. Five circulating endothelial populations were quantified by flow cytometry. Patients were divided in two groups of severity according to the median value of peak oxygen uptake (VO_2), predicted peak VO_2, ventilation (VE)/carbon dioxide output (VCO_2) slope and ejection fraction (EF).

Research results

Patients with lower peak VO_2 increased the mobilization of $CD34^+/CD45^-/CD133^+$, $CD34^+/CD45^-/CD133^+/VEGFR_2$, $CD34^+/CD45^-/CD133^-$ and $CD34^+/CD45^-/CD133^-/VEGFR_2$, while patients with higher VO_2 increased the mobilization of $CD34^+/CD45^-/CD133^+$, $CD34^+/CD45^-/CD133^+/VEGFR_2$, $CD34^+/CD133^+/VEGFR_2$, $CD34^+/CD45^-/CD133^-$ and $CD34^+/CD45^-/CD133^-/VEGFR_2$. A similar increase in the mobilization of at least four out of five cellular populations was observed after maximal exercise within each severity group regarding predicted peak, VE/VCO_2 slope and EF, as well ($P < 0.05$). However, there were no statistically significant differences in the mobilization of endothelial cellular populations between severity groups in each comparison ($P > 0.05$).

Research conclusions

Our study has shown an increased EPC and CEC mobilization after maximal exercise in CHF patients, but this increase was not associated with syndrome severity.

Research perspectives

EPCs could be the cornerstone to the treatment of CHF. Understanding their possible mechanisms of action on vascular endothelial function through exercise would create

innovative ideas regarding their distribution and proliferation in these patients in order to take advantage of their beneficial effects on the endothelium and reverse cardiac remodeling.

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