Original article

Coronary Disease Extension Determines Mobilization of Endothelial Progenitor Cells and Cytokines After a First Myocardial Infarction With ST Elevation

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A B S T R A C T

Introduction and objectives: Multivessel coronary disease is still a postinfarction prognostic marker despite new forms of reperfusion, such as primary angioplasty. The aim of this study was to determine the time sequence of various sets of endothelial progenitor cells and angiogenic cytokines (vascular endothelial growth factor, hepatocyte growth factor) according to the degree of extension of the postinfarction coronary disease.
Methods: We studied the release kinetics in 32 patients admitted for a first myocardial infarction with ST elevation, grouped according to whether they had single or multivessel disease, and 26 controls.
Results: The patients had a higher number of endothelial progenitor cells and angiogenic cytokines than the controls at all 3 measurements (admission, day 3, and day 7) of the following subsets: CD34, CD34+CD133+, CD34+KDR+, and CD34+CD133+KDR+CD45+(weak); this latter was higher on day 7. The levels of these cell subsets were all higher in the patients with single-vessel disease and at all 3 measurements. The vascular endothelial growth factor levels were raised during the first week and the hepatocyte growth factor showed an early peak on admission for infarction. No significant differences were seen in the cytokines according to coronary disease extension.
Conclusions: Although the release kinetics of different subsets of endothelial progenitor cells in patients with a first acute myocardial infarction with ST elevation was similar in those with single vessel disease to those with multivessel disease, the number of circulating endothelial progenitor cells was greater in the patients with single vessel disease. The vascular endothelial growth factor levels were raised during the first postinfarction week and the hepatocyte growth factor were higher on admission.

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La extensión de la enfermedad coronaria determina la movilización de las células progenitoras endoteliales y las citocinas tras un primer infarto de miocardio con elevación del ST

R E S U M E N

Introducción y objetivos: La enfermedad coronaria multivaso es un importante factor pronóstico postinfarto a pesar de nuevas formas de reperfusión como la angioplastia primaria. El objetivo del presente estudio es determinar la secuencia de variación de diferentes poblaciones de células progenitoras endoteliales y factores angiogénicos (factor de crecimiento endotelial vascular, factor de crecimiento hepatocitario) según el grado de extensión de la enfermedad coronaria.
Métodos: Estudiamos la cinética de liberación en 32 pacientes ingresados por un primer infarto, agrupados según tuvieran enfermedad coronaria monovaso o multivaso y 26 sujetos que constituyen el grupo control.
Resultados: Los pacientes presentaban un mayor número de células progenitoras endoteliales y citocinas angiogénicas que los controles en las tres determinaciones realizadas (al ingreso, día 3 y día 7) de las siguientes subpoblaciones: CD34, CD34+CD133+, CD34+KDR+ y CD34+CD133+KDR+CD45+ (débil); este último era mayor el día 7. Los valores de las tres poblaciones analizadas eran mayores en los pacientes con enfermedad coronaria monovaso en las tres determinaciones. Las cifras del factor de crecimiento endotelial vascular subían durante la primera semana y las del factor de crecimiento hepatocitario

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mostraron un pico precoz al ingreso. No apreciamos diferencias significativas en las variaciones de citocinas según el grado de extensión de la enfermedad coronaria.

Conclusiones: Aunque las cinéticas de liberación de diferentes poblaciones de células progenitoras endoteliales en pacientes con un primer infarto agudo de miocardio con enfermedad monovaso con enfermedad multivaso fueron similares, su número fue mayor en los pacientes con enfermedad coronaria monovaso. Las cifras del factor de crecimiento endotelial vascular ascendieron durante la primera semana y las del factor de crecimiento hepatocitario muestran un pico precoz al ingreso.

Abbreviations

EPC: endothelial progenitor cells
HGF: hepatocyte growth factor
VEGF: vascular endothelial growth factor

INTRODUCTION

Cardiovascular disease is the most frequent cause of death in the world. Of particular importance is ischemic heart disease, despite a slightly better prognosis in acute myocardial infarction in the last decade.[1] This situation necesitates improved understanding of the underlying pathophysiological mechanisms in acute myocardial infarction to be able to apply more effective treatments and thereby improve the ominous prognosis of this disease.

The endothelial progenitor cells (EPC) described by Asahara et al.,[2] derived from pluripotential hematopoietic precursors, are characterized by possessing a certain regenerating role in vasculogenesis and angiogenesis, shown in the surface markers CD34, CD133 and KDR, or VGFR; they have already been used in clinical trials.[3] Nevertheless, EPC are difficult to define, as diverse populations of progenitor cells may coexist in the circulation, all of which have been referred to as EPC by different authors, including CD34+CD133+KDR+ early EPC and CD34+CD133-KDR+ late EPC (after losing their CD133).[4] Recently, a few groups have defined the EPC phenotypically using an additional membrane marker, CD45+ (weak), together with the classical markers CD34+CD133+KDR+[5].

In addition to the important role played by various cell subsets, several cytokines have also been implicated; these are released as a result of vascular damage and induce the mobilization of EPC from bone marrow.[6] Of note among these cytokines are the vascular endothelial growth factor (VEGF)[6] and the hepatocyte growth factor (HGF),[7] suggesting their possible intervention in myocardial repair mechanisms. VEGF is involved in stem-cell-mediated cardiac repair, both because of its prominent role in angiogenesis and its capability of mobilizing bone marrow stem cells into the peripheral blood in myocardial infarction patients. HGF and its receptor (c-met) are involved in cardiogenesis, in which it is transiently expressed during early cardiac development. Both in vitro and in vivo, HGF enhanced survival of cardiomyocytes under ischemic conditions.[8]

The prognostic importance of multivessel coronary artery disease in patients with acute myocardial infarction is well known[9] and is related with various biochemical markers of cell damage, such as alpha-actin protein.[10] This prognostic association still remains despite the presence of new reperfusion methods for the treatment of acute myocardial infarction.[11]

In spite of the controversy concerning the kinetics of the rise in different cell subsets and cytokines, ie, increases with effect from the time of infarction[12–14] versus progressive increases reaching a peak one week after the infarction,[15] no study has yet examined these increases in relation to the degree of the extension of coronary disease, which occurs in half of all infarctions. Additionally, reperfusion therapy is the treatment used in clinical trials of cell implants[3] and in which we still do not know the optimum implant time for these cell subsets.

The aim of this study was to measure the temporal sequence of the release into peripheral blood of the mononuclear cell subsets CD34+, CD34+CD133+, CD34+KDR+, and CD34+CD133+KDR+CD45+ (weak) and the circulating cytokines VEGF and HGF according to the degree of extension of coronary disease, to attempt to determine the optimum time for postinfarction cell implantation. In addition, we studied differences in mononuclear cell subsets and circulating cytokines between a control group and patients with a first myocardial infarction.

METHODS

Patients

From August 2006 to June 2007 we studied 32 patients admitted with infarction, defined as acute ST-elevation myocardial infarction (STEMI) accompanied by suggestive chest pain and an elevation of at least 3 mm in the ST segment in at least 3 precordial leads with positive troponin, no history of ischaemic heart disease and within 8 h of symptom onset. A control group was composed of 26 persons with similar demographic characteristics who attended the hospital for blood tests. They provided a brief medical history concerning risk factors in order to exclude patients with possible cardiovascular symptoms. Exclusion criteria, for both the cases and the controls, included the presence of chronic ischemic heart disease, severe valve disease, a history of myocardial infarction, current or previous neoplasm, chronic use of anti-inflammatory drugs and chronic renal failure (creatinine >2 mg/dL). Another 13 controls with similar demographic characteristics were later used for the measurement of the control cytokines.

Single-vessel disease was considered to be present if any epicardial artery >2.5 mm had stenosis >70%; multivessel disease was considered to be the involvement of >1 epicardial vessel (coronariography were revised by 2 experienced interventional cardiologists). The study fulfilled the norms of the Declaration of Helsinki and was approved by the hospital ethics committee. All the patients gave written informed consent to participate in the study.

Isolation of Peripheral Blood Mononuclear Cells

Blood was drawn 3 times from the patients with STEMI (on arrival at the emergency department, and on days 3 and 7 afterwards) and once from the controls. The sample was processed and stored for later analysis by flow cytometry after verifying that all the patients had normal blood test results. The samples were all collected in ethylene diamine tetraacetic acid (EDTA) tubes and processed within 24 h of extraction. Blood

samples were diluted with phosphate-buffered saline and peripheral blood mononuclear cells were isolated by density gradient centrifugation with Ficoll separating solution. Recovered cells were washed twice with phosphate-buffered saline and resuspended in phosphate-buffered saline.

Immunophenotype Study and Quantification of Circulating Progenitor Cells

Quantification of the peripheral blood CD34+ cell population was done according to the ISHAGE guidelines.[16] The viable cells were stained with the following antihuman antibodies: anti-CD34 conjugated to fluorescein isothiocyanate (Becton Dickinson, Pharmigen, San Jose, California, United States), phycoerythrin-conjugated anti-CD133 (Miltenyi Biotech, Bergisch Gladbach, Germany), anti-CD45 conjugated to peridin-chlorophyll-protein (Becton Dickinson, San Jose, California, United States) and allophycocyanin-conjugated anti-KDR (R&D Systems, Minneapolis, Minnesota, United States). Isotype-matched (Becton Dickinson and R&D systems) controls were used to rule out the presence of nonspecific antibody binding. The cells were acquired with a FACScalibur flow cytometer and analyzed using Cell-Quest software (Becton Dickinson). The analysis was performed excluding cellular debris in a side scatter/forward scatter dot plot. Measurements were made of the absolute number of CD34+, CD34+CD133+, CD34+KDR+, and CD34+CD133+ KDR+ CD45+ (weak) cells in the circulating mononuclear cell population.

Measurement of Circulating Vascular Endothelial Growth Factor and Hepatocyte Growth Factor Levels

Peripheral blood was collected from patients in EDTA tubes on admission and on days 3 and 7 after the onset of the acute myocardial infarction. The patients were receiving no treatment with heparin that could have altered the VEGF values. The blood samples were centrifuged for 15 min at 1000 g within 30 min of collection, after which the plasma samples were immediately aliquoted and stored at −80 °C until assay. Plasma VEGF and HGF concentrations were measured in duplicate with 2 commercially available quantitative sandwich enzyme immunoassay kits (R&D Systems, Minneapolis, Minnesota, United States) according to the manufacturer's guidelines. Inter-assay (1 sample tested in 10 separate assays) and intra-assay variations (1 sample tested 10 times on 1 plate) were 7.1% and 4.2% for HGF, and 8.2% and 5.1% for VEGF, within the range of the precision of the assay given by the product information from R&D Systems Inc. The minimum detectable value was 9 pg/mL for VEGF and 40 pg/mL for HGF.

Statistical Study

The quantitative data are expressed as the mean ± standard deviation and the qualitative data as percentages. The Student *t* test was used to analyze the difference between continuous quantitative variables in 2 independent samples, provided normality was fulfilled, otherwise the Mann-Whitney U test was used. The Shapiro-Wilk test was used to verify normality. The Friedman test was used to analyze the differences in repeated measures, with the Wilcoxon test for paired samples. Bonferroni correction was applied to *P*-values, with significance set at *P*<.016. Analysis of differences between continuous quantitative variables in more than 2 independent samples was done by ANOVA (with the Bonferroni test to analyze differences between subgroups). Categorical variables were analyzed with the chi-square test or Fisher's test. We used a general

univariate linear model in the data analysis to control for selection bias of sex and smoking as variables in this case-control study. The correlations between the variables were measured with the Pearson correlation coefficient when the variables fulfilled the criteria of normality and the Spearman correlation coefficient when these criteria were not met. Differences were considered significant if *P*<.05. The data were analyzed with SPSS 12.0 (SPSS Inc., Chicago, Illinois, United States).

RESULTS

Baseline Characteristics

Of the 32 patients with an infarction, 8 were initially treated with primary angioplasty and the other 24 with fibrinolysis, though rescue primary angioplasty was required in 9 of these cases. Of the 15 patients who did not undergo coronary angiography during the acute phase, 10 did so during the following 5±2 days. The remaining 5 patients with uncomplicated inferior myocardial infarction did not undergo angiography either for clinical reasons or because they refused to consent. The ejection fraction (EF) was measured in all the patients, either during the hemodynamic study or after an echocardiographic study (4.2±1.5 days). On discharge, 87% of the patients were being treated with angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists, 87% with beta-blockers, 88.6% with statins, and 63.4% with the combination of acetylsalicylic acid and clopidogrel. No differences were found in previous vascular history (carotid, aorta, or iliac-femoral) or previous medication (antihypertensives, statins, and platelet antiaggregant therapy).

The baseline characteristics of the patients with STEMI differed from those of the controls in the percentage of patients who smoked (63.6% vs 15.4%) and distribution by sex (more women in the control group) (Table 1). No differences were found in the baseline characteristics of the patients according to the degree of extension of their coronary disease (Table 1).

Measurement of Cell Populations and Time Sequence

The initial blood sample was drawn a mean of 234.4±27 min after the onset of symptoms of STEMI, before starting treatment with fibrinolysis and heparin. All the cell subsets analyzed had higher values in the patients than the controls (Table 2, Fig. 1). The patients still had higher cell counts than the controls after controlling for the selection bias of sex and smoking in a univariate linear model.

The time sequence showed significant differences in the numbers of the different cell populations at admission, and on days 3 and 7 post-infarction (Table 2, Fig. 1). Analysis of the differences between groups showed higher numbers of all the cell subsets on day 7 compared to day 3, and on day 7 compared to the values on admission (*P*<.016). No differences were found in the numbers of CD34+, CD34+CD133+, or CD34+KDR+ cells on day 3 compared to the admission values, although the number of CD34+CD133+KDR+CD45+ (weak) cells was lower (*P*=.012) (Table 2). No differences were found in the numbers of the various cell subsets or in the cytokines at any of the 3 measurements according to the presence of postinfarction ventricular dysfunction (EF <45%), except for higher levels of VEGF at the second measurement in the patients with ventricular dysfunction (0.294 vs 0.22 ng/mL, *P*<.005).

Examination of the variations in cell subsets at the 3 time points considered (on admission, and on days 3 and 7) showed significant differences for each of the CD34+, CD34+CD133+, CD34+KDR+, and

Table 1

Baseline Characteristics of the Whole Patient Group, the 27 Patients Who Underwent Angiographic Study, and Controls

	STEMI (n=32)	STEMI Single vessel (n=15)	STEMI Multivessel (n=12)	Controls (n=26)
Age, years	59.5±12.4	58.6±11.4	60.9±13.9	64.2±6.4
Women, %	9 (28)[*]	3 (20)[*]	3 (25)[*]	15 (57.7)
Cardiovascular risk factors, %				
Hypertension	21 (65.6)	8 (53.3)	9 (75.0)	15 (57.7)
Smoking	21 (65.6)[*]	10 (66.7)[*]	10 (83.3)[*]	4 (15.4)
Diabetes mellitus	11 (34.4)	5 (33.3)	4 (33.3)	8 (30.8)
Hypercholesterolemia	14 (43.8)	5 (33.3)	5 (41.7)	11 (42.3)
Anterior infarction	13 (42.0)	7 (46.7)	4 (33.3)	
Thrombolytic therapy	24 (74.0)	12 (80.0)	8 (66.7)	
Time from pain onset to reperfusion, min	234±27	228±24.8	236±30.2	
Ejection fraction	51.9±12.2	52.5±13.4	50.6±12.7	
Use of abciximab	22 (68.7)	12 (80.0)	10 (83.3)	
Troponin T peak	54.4±13.1	61.6±12.2	77±13.6	

STEMI, ST segment elevation myocardial infarction.
The data are expressed as the total number and in brackets the percentage of the total of each group or as means ± standard deviation.
[*] Significant differences compared with the control group (*P*<.05).

Table 2

Immunophenotype/Characterization and Number (Mean ± Standard Deviation) of the Circulating Progenitor Cells and Cytokines in Peripheral Blood in Patients After ST-Elevation Myocardial Infarction

	Control	On admission	Day 3	Day 7
Number cells /10³ PBMCs				
CD34+	5.7±2.0	51.9±31.9[a]	51.5±32.3[a,b]	55.4±33[a,b]
CD34+CD133+	0.6±0.3	9.2±5.7[a]	8.5±5.2[a,b]	10.5±6.5[a,b]
CD34+KDR+	0.6±0.2	8.5±5.3[a]	8.2±5.3[a,b]	9.7±5.8[a,b]
EPC	0.5±0.2	6.8±4.5[a]	6.5±4.2[a,b]	8.0±5.2[a,b]
Cytokines, pg/mL				
VEGF	57.0±26.6	68.1±43.9	97.7±63.9[a]	91.5±49.3[a,b]
HGF	859.1±607.8	10 175.5±4561.3[c]	3743.6±1956.3[c]	2655.4±4023.9[a,b]

EPC, endothelial progenitor cells; HGF, hepatocyte growth factor; PBMCs, peripheral blook mononuclear cells; VEGF, vascular endothelial growth factor.
EPC defined as CD34+CD133+KRD+CD45+ (weak) cells.
[a] Significant differences compared with the control group (*P*<.05).
[b] Significant differences compared with the control group (*P*<.001).
[c] Significant differences (Friedman test) in repeated measurements (*P*<.001).

EPC cell subsets, with the EPC being significantly lower on day 3 than at admission (*P*<.016) and the CD34, CD34+CD133+, CD34+KDR+, and EPC being significantly higher on day 7 than on admission and on day 3 (*P*<.016).

Measurement of Cytokines and Time Sequence

The VEGF values were significantly higher on days 3 and 7 of the acute phase of the infarction than in the control group (97.7 and 91.5 vs 57 pg/mL) (Table 2). No significant differences were seen between the VEGF values on admission and those of the control group (68.1 vs 57) (Table 2). The HGF values were increased at all 3 time points during the acute phase of the infarction compared with the control group values (10 175, 3743, and 2655 vs 859 pg/mL) (*P*<.003). The first measurement was significantly higher than the second and third measurements (*P*<.016), with no significant difference between the latter two measurements (Table 2). Thus, the HGF values were higher during the acute phase of the STEMI compared with the control group values, with an early peak at the first measurement, on admission.

These results show that the two cytokines possess different release kinetics after an infarction. VEGF peaked on day 3, when it doubled the peripheral blood levels, and then declined progressively, but still showed high levels on day 7 (1.8 times those of the controls). HGF, however, had very high levels on admission after the infarction (11.8 times higher than those of the controls) and remained high during the whole of the first postinfarction week, though falling progressively to day 7 (3 times higher than the controls).

Cell Subsets, Cytokines and Time Sequence According to Degree of Extension of Coronary Disease (Single vs Multi-Vessel)

Significant differences were found between the various progenitor cell subsets at the 3 measurement points according to the degree of extension of coronary disease, as documented by coronary angiography (Table 3). Their levels were higher in the patients with single vessel disease. The release kinetics of the circulating progenitor cell subsets did not vary according to the degree of coronary involvement.

No differences were found between the single vessel and the multivessel groups in the values of the 2 cytokines studied, with similar release kinetics in each group (Fig. 1). Nor were differences found between patients treated with fibrinolisis and primary angioplasty, even at the third measurement when there was vascular damage due to stent implantation.

Correlations Between the Number of CD34 Cells and the Subsets; Levels of Cytokines (Vascular Endothelial Growth Factor and Hepatocyte Growth Factor) and Degree of Extension of Coronary Disease (Single vs Multi-Vessel)

None of the correlations between the number of cells in the different subsets analyzed and the levels of VEGF and HGF at
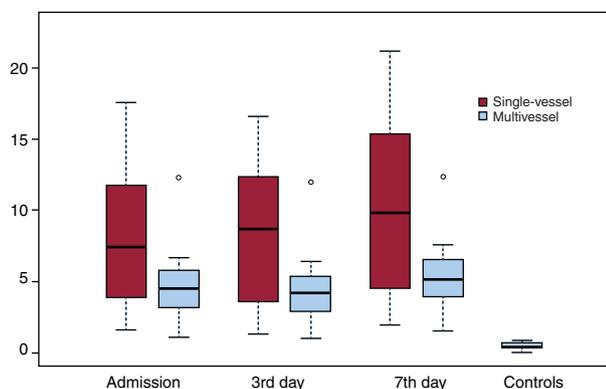
Figure 1. Kinetics of endothelial progenitor cell according to the extension of coronary artery disease.

the 3 measurement points (on admission and on days 3 and 7) were significant (Table 4). Nor were significant correlations found between the levels of the cells or cytokines and the CK peak or the EF <45% (Table 4). No significant differences were found in the correlations of the cell subsets and the cytokines with the presence of single or multivessel disease in the patients who underwent coronary angiography (Data not shown) nor in the difference in the numbers of the cells at the 3 measurement points. Neither were differences detected in the levels of the cells and cytokines and the kinetics in the patients treated with primary angioplasty as compared with fibrinolysis, nor in the subgroups with single vessel or multivessel disease (data not shown).

DISCUSSION

The main finding of this study was the higher number of peripheral blood EPC in patients with infarction and single vessel disease compared with patients with infarction and multivessel disease, as documented by coronary angiography. The release kinetics of these cells in both these subgroups was higher on day 7 postinfarction, with no significant differences in the release kinetics of the 2 cytokines studied (VEGF and HGF) during the first postinfarction week.

A major ischemic event releases EPC from the bone marrow and chemoattractant factors (cytokines) in an attempt to "repair" the ischemic insult.[2,16] Our results showed a very marked increase in the various CD34+ cell subsets, including CD34+ CD133+ and KDR+CD45+ (weak) (10 times the normal value) and the angiogenic cytokines VEGF and HGF during the first week after

an infarction, as compared with a control group. This rise was very early (within 4 h of symptom onset) and the highest values for the cell subsets were found on day 7 after symptom onset. These data indicate that adequate regulation of signaling between the bone marrow, the peripheral circulation, and the infarcted myocardium is important in orchestrating the process of mobilization, homing, incorporation, survival, proliferation, and differentiation of stem cells, leading to myocardial regeneration.[17]

More detailed analysis of the cytokines showed that VEGF was increased with effect from the second measurement (day 3) and that HGF showed an early release peak at the first measurement (within 4 h of symptom onset), remaining high compared to the control group, but falling gradually during the first postinfarction week. After tissue injury, when formation of new blood vessels is urgently required, VEGF mediates proliferation, differentiation, and chemotaxis of endothelial cells. The early postinfarction rise in HGF would be in response to the initial requirement of the organism for factors inducing cell survival, antiapoptosis, improvement in the survival of cardiomyocytes under ischemic conditions, and a cell proliferation stimulus,[8] all of which have been described as factors associated with HGF. Later, after the ischemic insult has become established (days 3-7), the myocardium attempts to favor the formation of microvessels, an angiogenic stimulus that is produced via the VEGF.[18]

The EPC decrease under certain chronic conditions, in the presence of cardiovascular risk factors, and in older persons[18]; a lower capacity to produce colony-forming units has even been seen in patients with chronic coronary disease and multivessel lesions.[19] However, despite the development of cell therapy in postinfarction patients, the release kinetics of circulating progenitor cells and peripheral blood cytokines is unknown, as is their relationship to the degree of extension of coronary disease in patients with a recent infarction. This information could help decide which patients could benefit from this therapy, and at what particular time. The lower number of EPC in the patients with multivessel disease could be the consequence of the organism's loss of capacity for vascular regeneration and the release of a sufficient number of EPC, described in patients with arteriosclerotic disorders.

Concerning the kinetics of the 2 angiogenic cytokines, the VEGF levels rose from day 3 in the patients with single-vessel disease and in those with multivessel disease, with no differences between the groups. Significant differences were found, however, in the patients with single-vessel disease compared to the controls. Nevertheless, after an early peak in the levels of HGF on admission, the levels remained stable on days 3 and 7 in both groups compared with the controls. The level of significance compared

Table 3

Immunophenotype/Characterization and Number (Mean ± Standard Deviation) of the Circulating Progenitor Cell Subsets and Cytokines in Peripheral Blood in Patients After ST-Elevation Myocardial Infarction

	Control	Single vessel			Multivessel		
		Admission	Day 3	Day 7	Admission	Day 3	Day 7
Number cells/10³ PBMCs							
CD34+	5.7±2.0	62.68±39.8[a,b]	60.9±39.7[a]	68.8±40.6[a,b]	37.0±17.9[a]	38.1±18.8[a]	38.3±16.8[a]
CD34+CD133+	0.6±0.2	10.8±6.8[a]	10.1±5.6[a]	12.9±7.8[a,b]	7.1±3.8[a]	6.4±3.4[a]	7.8±3.7[a]
CD34+KDR+	0.6±0.2	10.0±6.2[a]	9.2±5.7[a]	11.7±6.8[a]	6.6±3.5[a]	6.2±3.5[a]	7.3±3.4[a]
EPC	0.5±0.2	8.8±5.5[a,b]	8.2±4.9[a,b]	10.6±6.2[a,b]	4.8±2.8[a]	4.6±2.9[a]	5.6±2.7[a]
Cytokines, pg/mL							
VEGF	57.0±27.6	68.8±37.4	98.6±59.2[c]	96.1±42.5[c]	62.7±57.8	105.3±83.6	79.6±66.7
HGF	859.1±607.8	10 001.0±4560.8[a]	3037.4±4228.4[a]	1993.6±2332.2[c]	10 306.44±3603.8[a]	4039.22±3895.6[c]	3396.9±5989.8[c]

EPC, endothelial progenitor cells; HGF, hepatocyte growth factor; PBMCs, peripheral blook mononuclear cells; VEGF, vascular endothelial growth factor.
Significant differences (Friedman test) in repeated measurements ($P<.001$) in different cell types subsets in single and multivessel patients.
EPC defined as CD34+CD133+KRD+CD45+ (weak) cells.
[a] $P<.05$ vs control group.
[b] $P<.001$ vs control group.
[c] $P<.05$ vs multivessel group.

Table 4

Correlations Between the Endothelial Progenitor Cell Subsets, Cytokines, Ejection Fraction and Creatine-Phosphokinase Peak

	VEGF			HGF			EF			CK peak		
	Admission	Day 3	Day 7	Admission	Day 3	Day 7	Admission	Day 3	Day 7	Admission	Day 3	Day 7
CD34	0.24	−0.15	−0.12	−0.31	−0.27	0.30	0.07	0.04	0.08	0.13	0.14	0.15
CD34133	0.05	−0.18	−0.13	−0.19	−0.22	0.22	0.13	0.11	0.14	0.07	0.10	0.15
CD34KDR	0.03	−0.14	−0.12	−0.21	−0.21	0.24	0.10	0.08	0.15	0.07	0.09	0.06
EPC	−0.08	−0.30	0.00	−0.03	−0.16	0.12	0.23	0.17	0.25	0.18	0.17	0.04
VEGF							0.14	0.37*	−0.13	−0.17	−0.41*	0.14
HGF							0.22	−0.10	−0.11	−0.22	−0.10	0.22

CK, cytokines; EF, ejection fraction; EPC, endothelial progenitor cells; HGF, hepatocyte growth factor; VEGF, vascular endothelial growth factor.

* P<.05.

with the control group was greater in the patients with multivesel disease. It is known that HGF induces angiogenesis independently of VEGF,[20] and acts in synergy with VEGF for the amplification of angiogenesis. Our results suggest that the HGF levels are maintained over time in the patients with a greater extension of coronary disease in order to stimulate the release of EPC to the peripheral blood in patients whose EPC levels are clearly reduced. Likewise, the fact that there were no significant differences in VEGF levels compared with the controls could have been compensated by a greater production of HGF in those patients with greater vascular involvement. Furthermore, this greater increase could be related with the capacity of the HGF to induce proliferation not only in the mobilized EPC but also in the resident endothelial cells[21] and to improve the survival of the cardiomyocytes in the patients with multivessel coronary artery disease.

Like others,[6,11] we found no correlation between the numbers of the various cell subsets and the cytokines examined. Differences in the number of persons studied, the type of study population (unselected, treated with reperfusion options different from our study) and a marked interindividual variation in the number of cells, as described by Leone et al.,[13] may explain this discrepancy. Nor did we detect an association between the number of cells and cytokines with the left ventricular EF, again like others,[12] although some studies suggest that the greater the increase in mononuclear cells the better the recovery of ventricular function,[13] while others have found an association between the number of mononuclear cells and improved ventricular function, suggesting a beneficial effect of these cells.[13]

The clinical value of this study is relevant because it shows differences in the levels of EPC between subgroups of patients with a first infarction depending on the degree of extension of coronary disease, and the importance the HGF may have in the response of the organism to a greater extension of coronary artery disease. These findings might help clarify one of the aspects currently under debate in cell therapy, ie, the type of cells to use and the best time for implantation.[22–24] Unlike mesenchymal progenitor cells, whose release seems to be very early, the release of CD34 progenitor cells is more sustained over the first week and may even be greater on the seventh day, as reported here, which might suggest a benefit of late cell implantation (5th to 7th day), as reported in the REPAIR-AMI clinical trial.[25] The results of this study also show that the number of cells required to achieve a clinically relevant effect is greater in patients with multivessel disease. Of note is the clinical importance of HGF to stimulate the repair of the lesion and a possible relationship with the prognosis of patients with multivessel disease; this should be further examined in future studies.

Limitations

Despite the number of patients in this study, we noted clear differences in the numbers of cells according to the degree of extension of coronary artery disease in patients with a first acute myocardial infarction. We chose a first infarction to avoid bias in the number of circulating progenitor cells from a previous ischemia. We are unaware of the potential mechanism of previous ischemia in patients with multivessel disease. A possible limitation concerns differences in the baseline characteristics regarding sex and smoking despite use of a univariate linear model. This study provides a possible explanation for differences between single-vessel and multivessel coronary disease. Other studies will be required to determine the efficacy of this idea.

CONCLUSIONS

Our results show the mobilization of a higher number of peripheral blood progenitor cells in patients who have a first acute myocardial infarction and single-vessel disease compared with patients with a first infarction and multivessel disease, as documented by coronary angiography. The release kinetics (VEGF, HGF) in both subgroups of patients was greater on day 7 after the infarction. HGF had an early peak, within 4 h of symptom onset, and higher values on days 3 and 7 in those patients with multivessel acute coronary disease, suggesting the relevant role of HGF in the proliferation and repair of cardiac tissue in these patients.

CONFLICTS OF INTEREST

None declared.

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