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Short Communication

Mechanism of Ventricular Volume Improvement after Autologous Stem Cell Implantation in Acute Myocardial Infarction

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We tried to obtain a significant number of CD133+ and CD34+ haematologic precursors from systemic circulation by aphaeresis, and analyzed necrosis area reduction by Tc-91 myocardial perfusion scanning and left ventricular diameters by cardiac ultrasound in a group of patients who received stem cell therapy in the first week after a myocardial infarction.

Materials and Methods

The study was designed as a blinded randomized trial, it was approved by the local government ethics committee (CEIC-IB) and the Spanish Medicines Agency.

Patients: We selected for blood aphaeresis patients aged 18 to 80 years old, presenting with their first STEMI in Killip class I to III. All patients underwent a successful primary coronary angioplasty in the first 6 hours of the infarction initiation, and followed the standard medical treatment which included double antiplatelet regimen, high dose statins, betablockers and ACE inhibitors.

Cell collection: Twelve patients were randomized to cell aphaeresis. They received G-CSF (Filgastrim 3µg/Kg/day) in the first days after the infarction and always 2-3 before the aphaeresis procedure. Cell collection was performed by means of the cell selector Haemonetics MCS+™ (Haemonetics, Braintree, Ma, USA). Haemonetics MCS+™ cell selector is a mobile, bedside, intermittent flow selector. A single aphaeresis procedure was done. Then, a sample was collected to perform cell count, particularly CD34+ and CD133+ cells by flow cytometry using 7ADD dye following the ISHAGE protocol [1]. If the patient had more than 1 x 10^6 CD133+ cells per 1500 x 10^6 total white cells were collected, the patient was included in the study.

Cell infusion protocol: The aphaeresis product was injected in the targeted heart region by means of a coaxial balloon angioplasty catheter during 3 minute periods of flow interruption by balloon inflations of an angioplasty balloon positioned inside the stent implanted at the lesion responsible of the heart attack.

Follow-up: Patients had a basal ultrasound exam as well as a maximal exercise myocardial perfusion test. Patients had clinical follow-up controls at one, three, six months and one year after cell implantation. The ultrasound exam and the perfusion test were repeated at one year. Transthoracic cardiac ultrasound and exercise myocardial perfusion tests were performed in the standard fashion and myocardial necrosis area was measured by the Manufacturer's quantification software [2,3]. Doctors performing the exams were blinded to the treatment group.

Statistical analysis tests used were Student t-test for paired samples, Chi-square test for quantitative variables, and linear regression with correlation coefficient for related variables.

Results and Discussions

Peripheral blood stem cell yield

We performed the collection procedure in 12 patients (3 women, 9 men). Mean age was 60.8 years (range 47 - 79), mean weight was 81.3 kg (99-45), and mean volemia was 5178.9 ml (4288 - 6441).

A mean aphaeresis product sample of 56 ± 4.8 ml was collected (Table 1). A per-protocol, pre-specified CD133+ cell number above 1 $x10^6$ in 1500 $x10^6$ total white cells was achieved in 8 patients (66.6%).

Data on white blood cells (WBC) number, as well as CD133+ and CD34+ cells are shown in table 1. We collected 3.1 to 8,8 x 10^9 (mean 5.4±1.7 x 10^9) WBC, which included 0.7 to 25.1 x 10^6 (7.4±7.6 x 10^6) CD133+ cells, which is a good cell yield, considering that we employed a G-CSF dose considerably inferior to what has been reported in the literature [4,5].

Infarct area reduction and ventricular remodeling

Out of these 8 patients with pre-specified adequate cell number, we excluded another 3 because: re-infarction (1 patient), lost to follow-up (1 patient) and three vessel disease (1 patient). Finally a group of 5 treated and 5 1:1 randomized controls composed the study group. Mean age was 59,4 ± 10,3 years and two of them were women (Table 2). We injected up to 1,298 x 10^6 WBC, of which 1.33 ± 0.73 $x10^6$ were CD133+ cells and 3.32 ± 1.27 x 10^6 were CD34+ cells (Table 1).

Infarct area regeneration: Both groups had a similar basal percentage area necrosis in the perfusion scan (Table 2). There was a significant decrease in necrosis area in treated patients at one year (23.4 ± 8.4 to 17.2 ± 7.4%; p<0.05). Controls showed no change in necrosis area at one year (30.6 ± 20.9 to 31.6 ± 21.4%;p=ns)(Figure 1).

Ventricular dimensions: Treated and control patients had similar basal end-systolic diameter (ESD) and basal end-diastolic diameter (EDD) (Table 2). After one year, ESD decreased in treated patients, but significantly increased in the control ones (Figure 1 a and b), showing a significant difference between both groups (33.2 ± 6.8 mm vs 42.6 ± 6.1 mm: p=0.044) and a very significant difference in ESD increment at one year between treated and control patients (-5.2 ± 5.3 mm vs 11.6 ± 3.5 mm; p<0.01). EDD did not increase in treated patients, while it showed a significant increase in non treated patients (Figure 1 a and b). When compared to stem therapy patients, controls also showed a significant difference at one year (48.8 ± 7.2 mm vs 61.2 ± 3.7 mm; p<0.01) and very significant increase in the EDD increment at one year follow up (-4.8 ± 6,2 vs 11.8 ± 2,9 mm; p<0.01).

Left ventricular fractional shortening: There were no differences in basal fractional shortening (FS) between treated and control groups (Table 2). It slightly decreased in controls and slightly improved in stem therapy patients (Figure 1 a and b). One year FS increment showed a trend towards an improvement in treated patients (4 ± 9 vs -6 ± 9; p=ns).

Left ventricular remodeling: ESD and EDD showed a significant increase in control patients at one year follow-up, while FS was preserved or slightly decreased (ventricular remodeling) (Figure 1 a and b). Treated patients had no change in ventricular volumes and showed a trend towards an improvement in FS. (Figure 1 and b).

Relationship between infarct area reduction and the prevention of left ventricular remodeling: Percentage necrosis area reduction was related to ESD increment (R=0.58;p<0.05) (fig 2a). The figure clearly shows that all treated patients (black dots) are located in the left inferior quadrant indicating an improvement in their ventricular function and myocardial recovery (necrosis area and ESD reduction). Controls (grey dots) are located in the superior half of the chart and around the zero value of the increment in necrosis area value, showing no myocardial regeneration and invariable ESD increases.

Necrosis percentage area reduction also had a significant relationship to EDD one year increment (R=0.68;p<0.05) (fig. 2b). In most patients who experience a reduction in infarct area, there was a concomitant reduction in LV size.

Peripheral progenitor cell protocol

Cell source and selection method: Autologous bone marrow mononuclear cells were used in most trials. We used mobilized haematopoietic progenitor cells from peripheral blood, which is a similar cell product. The advantage of peripheral blood aphaeresis is that it is less invasive, it is well tolerated, it requires no cell manipulation, and the aphaeresis product is

Patient	Aphaeresis volume (ml)	WBC ($x10^6$)	CD34+ Cells $x10^6$ in 1000 x 10^6 WBC	CD34+ cells injected ($x10^6$)	CD133+ Cells $x10^6$ in 1000 x 10^6 WBC	CD133+ cells injected ($x10^6$)
1 INCLUDED	**63**	**5260**	**3,06**	**2.24**	**1,37**	**1**
2 INCLUDED	**53**	**5330**	**2,49**	**2.78**	**0,9**	**1**
3 (no cells)	57	3960	1,16	-	0,43	-
4 (Re-infarction)	48	3880	1,57	-	1,57	-
5 (Lost Follow up)	56	4490	1,78	-	1,2	-
6 (no cells)	52	3180	1,6	-	0,22	-
7 INCLUDED	**59**	**7970**	**3,78**	**3.83**	**2,6**	**2,6**
8 (3-vessel disease)	66	6400	6,84	-	3,94	-
9 (no cells)	57	3990	1,28	-	0,43	-
10 INCLUDED	**55**	**5340**	**4,1**	**5.32**	**0,77**	**1**
11 (no cells)	57	6070	1,24	-	0,59	-
12 INCLUDED	**53**	**8850**	**2,07**	**2.46**	**0,84**	**1**
MEAN	**56**	**5390**	**2,58**	**3.32**	**1,22**	**1.33**
SD	**4.8**	**1710**	**1,66**	**1.27**	**1,08**	**0.73**

Table 1. Final cell count and cell yield of peripheral blood aphaeresis.

infused shortly after cell collection, thus avoiding cell quality deterioration. In addition to reduce cell quality, cell manipulation is not free of severe complications [6].

Cell number: There is a clear relationship of CD34+ cell number injected to myocardial recovery [7]. That phase I study showed that there is a linear relationship between the number of CD34+ cells injected and a favorable change in infarct size, which begins to work when more than 0.5 x 10^6 CD34+/ SDF-1 mobile cells were injected. We injected to our patients between 2.5 and 5 x 10^6 CD34+ cells (Mean = 3.32 ± 1.27) and also observed a reduction of infarct size by SPECT and of left ventricular dimensions one year after the heart attack.

Prevention of left ventricular remodeling: Post myocardial infarction left ventricular remodeling consists of an increase in ventricular volumes directed to maintain systolic ejection volume. Several papers have found a preservation of ventricular volumes in stem cell treated patients [8,9]. In our study, cell therapy patients showed a decrease in necrosis area while controls had no regeneration of the necrotic zone. Then, control patients show an increase in both, ESD and EDD while FS remains unchanged one year after myocardial infarction (ventricular remodeling). But patients treated with intracoronary autologous cell infusion had no change or even an improvement in terms of a decrease in left ventricular diameters one year after the heart attack. It also evident, that treated patients had a decrease of percentage infarct area measured by Tc-99m gammagraphy which is related to the change in ventricular diameters: patients who have a decrease in necrosis area show a parallel decrease in ventricular diameters and those in whom necrosis area does not improve, show an increase in ventricular diameters which leads to ventricular remodeling and heart failure (Figure 2). The functional improvement produced by cell injection is basically related to a regeneration of the infarcted area which leads to a preservation of ventricular diameters as seen in figure 2.

Limitations of the study: The present study main limitation is the number of patients included. Thus, its clinical application awaits further and broader investigations. However, the proof of concept that bone marrow cells obtained from peripheral blood shortly after myocardial infarction improve myocardial recovery has been established.

	Treated (n=5)	Controls (n=5)	p
Age (years)	55.8 ± 6.6	63 ± 11.5	**n.s.**
Male (%)	3	5	**n.s.**
Smokers (%)	2	5	**< 0.05**
Diabetes (%)	1	0	**n.s.**
Hypertension (%)	3	2	**n.s.**
Hipercholesterolemia (%)	3	3	**n.s.**
Anterior MI (%)	3	4	**n.s.**
Killip > I (%)	2	2	**n.s.**
Time to PCI (minutes)	54 ± 21.1	60 ± 21.1	**n.s.**
Time to cell infusion (days)	5.8 ± 1.17	-	**.**
EF angio	51 ± 8.1	52 ± 9.5	**n.s.**
Necrosis Area (%)	23.4 ± 8.4	30.6 ± 20.9	**n.s.**
ESD (mm)	38.4 ± 6.7	31 ± 4.9	**n.s.**
EDD (mm)	53.6 ± 9.8	49.4 ± 5.5	**n.s.**
FS (%)	28 ± 9	37 ±8	**n.s.**
Betablockers (%)	100%	100%	**n.s.**
ACE inhibitors (%)	40%	60%	**n.s.**
Statins (%)	80%	80%	**n.s.**

Table 2. Patient demographics. PCI: Percutaneous Coronary Intervention. MI: myocardial infarction, EF: ejection fraction, ESD end-systolic diameter, EDD end-diastolic diameter, FS: fractional shortening.

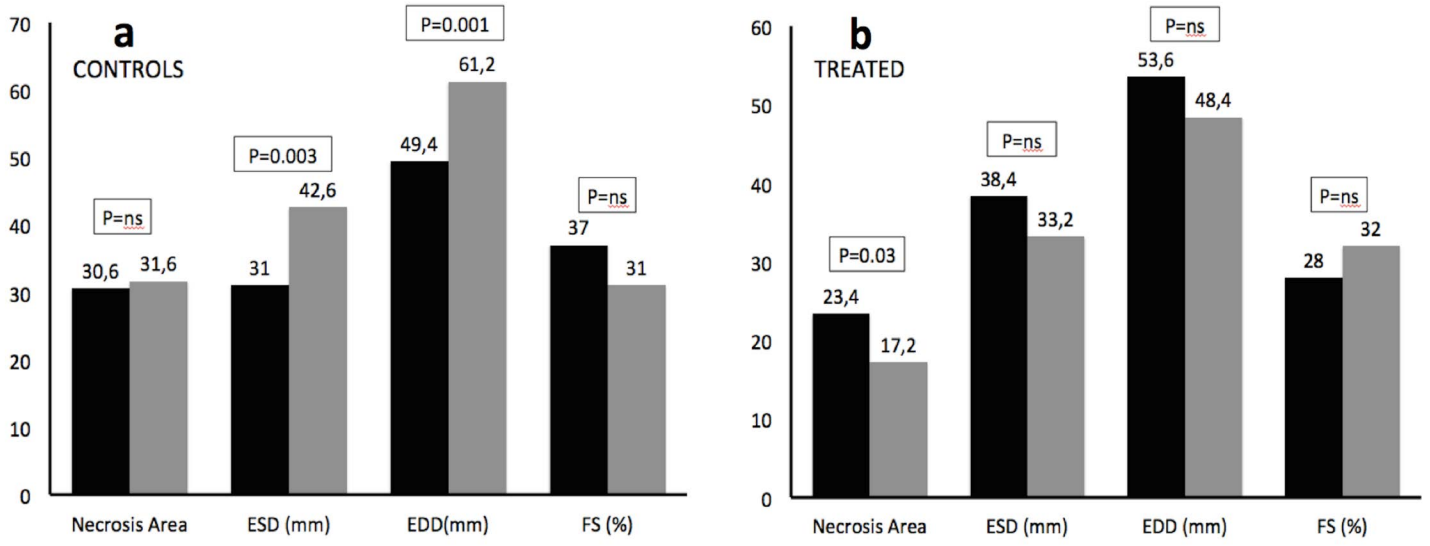

Figure 1: a. Control patients. **b** Treated patients. Basal (black columns) and one-year follow-up measurements (grey columns). Necrosis area: percentage necrosis area at perfusion scan. ESD: End-systolic diameter. EDD End-diastolic diameter. FS: Fracional shortening.

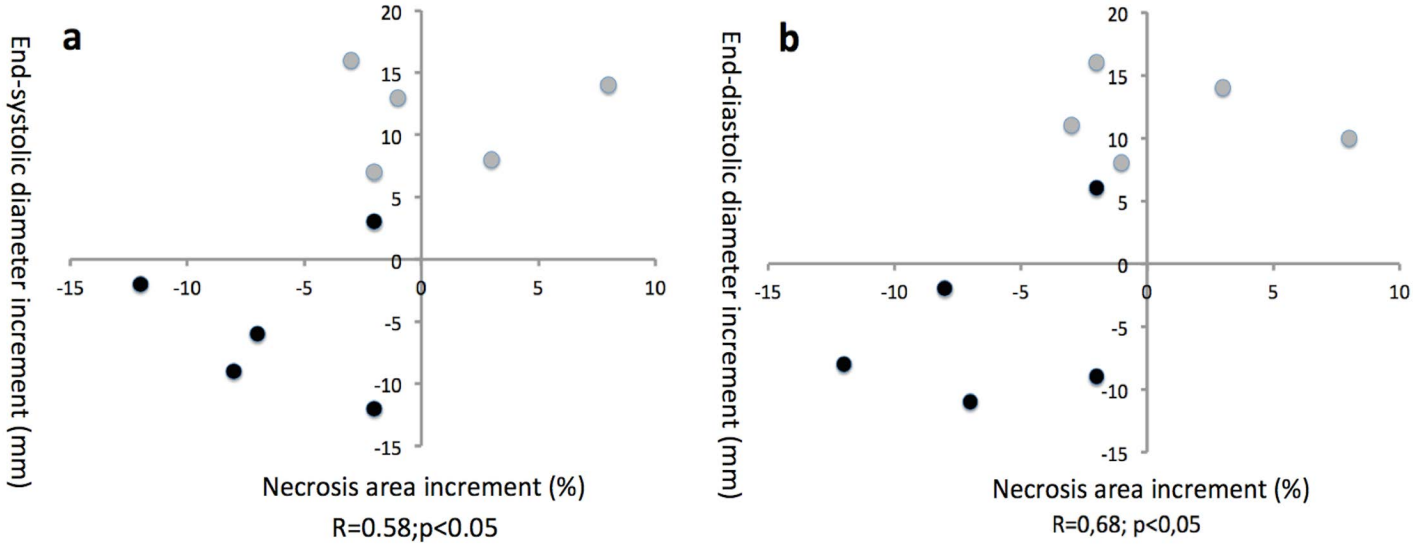

Figure 2: a. Necrosis area increment to end-systolic diameter increment at one-year follow up relationship. Grey dots: control patients. Black dots: treated patients. **b.** Necrosis area increment to end-diastolic diameter increment at one-year follow up relationship. Grey dots: control patients. Black dots: treated patients.

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