ESC HEART FAILURE
ESC Heart Failure 2020; **7**: 2962–2971
Published online 14 August 2020 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.12911

ORIGINAL RESEARCH ARTICLE

Intracoronary injection of haematopoietic precursor cells regenerates the borders, but not the core, of old myocardial scars

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Abstract

Aims Cell therapy regenerative potential is hindered by cell access to the infarct zone. We studied function recovery at the scar zone and its impact in global left ventricular function after intracoronary injection of haematopoietic precursor cells.

Methods and results Haematopoietic precursor cells were obtained by blood apheresis in patients with an old myocardial infarction, and the presence of CD34+ and CD133+ cells was quantified. Left ventricular function, volumes, and infarct zone segmental motion were measured by magnetic resonance imaging (MRI) and echo left ventricular segmental strain (LVSS). The aphaeresis product was administered to 20 patients in the coronary artery responsible for the myocardial infarction. High cell yield in blood aphaeresis product allowed us to inject a high number of cells in most patients. Three patients were excluded because of insufficient CD133+ cell number, and one more patient was excluded because of artefacts in MRI images. The remaining 16 patients were compared with 16 controls. After 1 year, infarct zone reduction was related to the number of CD133+ (R = 0.53; P %3C 0.05) and CD34+ (R = 0.63; P %3C 0.01) cells injected. The number of CD133+ cells injected was also related to an improvement in LVSS (R = 0.62; P %3C 0.01). In turn, scar zone reduction was related to an improvement in LVSS (R = 0.64; P %3C 0.01). End-diastolic volume showed a reduction at follow-up in the treated group when compared with control patients. MRI infarct area segments systolic thickness increase improved after treatment in treated patients [expressed as median (interquartile range)] [0.42 (−0.38 to 1.14) vs. 1.06 (−0.10 to 2.12) mm; P %3C 0.01], but not in controls [2.02 (0.75 to 3.4) vs. 1.91 (0.77–3.17) mm; P = not significant (n.s.)]. In cell therapy patients, the borders of the infarct zone, but not the core, showed a significant recovery [proximal rim: 0.48 (−0.18 to 1.33) vs. 1.07 (0.22–2.40) mm; P %3C 0.05, distal rim: 0.75 (0.26–1.40) vs. 1.76 (0.65–2.86) mm; P %3C 0.05, and core: 0.36 (−0.33 to 1.20) vs. 0.60 (−0.18 to 1.62) mm; P = n.s.]. That improvement was not observed in the control group [proximal rim: 1.20 (0.33–2.53) vs. 0.82 (−0.13 to 1.65) mm; P = n.s., distal rim: 1.24 (0.80–1.72) vs. 0.96 (0.19–1.81) mm; P = n.s., and core: 0.30 (−0.42 to 1.64) vs. 0.07 (−0.60 to 1.20) mm; P = n.s.]. Only small size infarcts showed a complete recovery in the cell therapy patients [systolic thickness increase post-treatment increment in infarcts ≤6 segments vs. >6 segments affected: 0.28 (−0.19 to 0.71) vs. −1.21 (−2.60 to −0.53) mm; P %3C 0.01].

Conclusions Intracoronary injection of peripheral blood-derived haematopoietic precursor cells produces a complete recovery of the borders and partial regeneration of the infarct core, which is directly related to the number of CD133+ and CD34+ cells injected. Cell therapy infarct zone regeneration prevents ventricular remodelling by preserving segmental contractility and halting left ventricular dilatation.

Keywords Heart repair; Heart regeneration; Myocardial infarction; Cell-based therapy

Received: 18 March 2020; Revised: 19 June 2020; Accepted: 8 July 2020
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Introduction

Children and adolescents with myocardial infarction due to anomalous origin of the left coronary artery arising from the pulmonary artery show a nearly complete regeneration when studied by magnetic resonance imaging (MRI), years after the event.[1] The body has the potential to self-regenerate damaged areas by means of resident stem cells or circulating blood haematological precursor cells. Unfortunately, heart muscle has a limited capacity to regenerate after injury, leading to ventricular remodelling and function deterioration.

Experimental studies performed in the early 2000s showed that bone marrow cells injected in the borders of the infarct area induced the regeneration of a great portion of the damaged area by newly formed myocytes and neo-vessel formation.[2] There is experimental evidence that blood pluripotent cells colonize the rim of the infarct zone and that this presence of pluripotent cells coincides with an improvement in angiogenesis and myocyte mitosis. Namely, CD34+ and CD133+ have shown to reduce infarct size and improve left ventricular function.[3–5] Moreover, a meta-analysis of cell therapy studies shows that intracoronary or intramyocardial cell injection reduces enlarged ventricular volumes in 7–17 mL and improves ejection fraction in 2–5 percentage points.[5]

Bone marrow is a heterogeneous tissue in which less than 1% of cells carry tissue regeneration capacity.[6] Cell selection is difficult and carries the risk of cell mutation, viability impairment, and death.[6] In addition, bone marrow puncture is an aggressive technique, not feasible in all patients. Blood apheresis is a much less invasive technique to obtain blood precursor cells. In this experiment, we tried to obtain a significant number of CD133+ and CD34+ haematologic precursors from systemic circulation by apheresis, to analyse necrosis area reduction and ventricular function recovery by MRI and echo left ventricular strain in a group of patients who received cell therapy several years after a myocardial infarction episode.

Methods

The study complies with the Declaration of Helsinki, was approved by the Regional Government Ethics Committee (CEIC-IB), the Spanish Medicines Agency (AEMPS), and the European Medicines Agency, and is registered under the number 2006-001772-20 in EudraCT as a randomized controlled trial.

Patients

The inclusion criteria were age %3E18 and %3C80 years old, myocardial infarction >3 years old with Q wave in >2 leads, acute myocardial infarction treated with primary angioplasty, Killip class I–III, complete revascularization with no obstructive coronary stenoses in coronary angiography, akinesia/dyskinesia area in at least two sequential segments at the infarct area without muscle viability signs in MRI, and signed an informed consent.

Forty patients were selected from our primary angioplasty database. They all had a myocardial infarction >3 years before inclusion and were followed by standard medical treatment that included antiplatelet agents, statins, beta-blockers, and angiotensin-converting enzyme inhibitors. They were in stable clinical condition, without inducible ischaemia in the treadmill test, and no significant lesions in coronary angiography. They underwent simple 1:1 randomization by a computer-generated random table.

All patients had a basal and 1 year follow-up complete blood test, cardiac MRI exam, ultrasound exam, and 24 h electrocardiogram (ECG).

Cell collection

Patients received G-CSF (filgrastim 3 μg/kg/day) 2–3 days before the apheresis procedure. Cell collection was performed by means of the cell selector Haemonetics MCS+TM (Haemonetics, Braintree, Ma, USA). Haemonetics MCS+TM cell selector is a mobile, bedside, intermittent flow selector. A single aphaeresis procedure was performed. Then a sample was collected to perform cell count, particularly CD34+ and CD133+ cells by flow cytometry using 7ADD dye following the ISHAGE protocol.[7]

Cell infusion protocol

Blood apheresis product remained at room temperature at the cath lab, while product samples were analysed in the cell lab.

After cell count and characterization were performed, the total number of cells to be injected was decided based on (i) the maximal total cell number to be injected and (ii) the minimal number of CD133+ cells (1×10^6) to be included in the study. Patients who had more than 1×10^6 CD133+ injected cells were included in the study.

A complete left cardiac catheterization procedure was performed with angiographic views of all arterial segments. We performed 3 min periods of flow interruption by angioplasty balloon inflations with the balloon positioned inside the stent implanted at the arterial segment responsible for the heart attack. The aphaeresis product was injected during the inflations in the targeted heart region by means of the coaxial angioplasty balloon catheter.

DOI: 10.1002/ehf2.12911

Magnetic resonance imaging

Images were obtained with a 1.5 T MR scan (SIEMENS Symphony-TIM) at baseline and 1 year after. Multiphase cardiac datasets with full left ventricular coverage were acquired using standard protocols. Scans were analysed in blinded fashion by a 10 year experienced radiologist in cardiac imaging using dedicated software (SyngoVia, SIEMENS). Standard left ventricular parameters were measured (ejection fraction and volumes), as well as the infarct mass and volume identified by late gadolinium enhancement sequences.

Magnetic resonance imaging left ventricular segments

Left ventricular segmentation was performed using the 16-segment polar display. Left ventricular segments functional analysis was performed by measuring the systolic increase in left ventricular wall thickness. Infarct zone functional analysis was performed by measuring the systolic increase in left ventricular wall thickness of the segments included in the MRI determined scar zone. Measurements were performed before treatment and at 1 year follow-up. Then we compared basal and 1 year data to assess the treatment effect.

Scar core, and proximal and distal rim

The definition of proximal rim, core, and distal rim of the scar zone was performed naming the central scar segments in the bullseye view that were not in contact with non-infarcted segments, as 'core'. The peripheral segments were named as 'proximal' and 'distal' in relation to their location in the polar display.

Small infarcts

The range of the infarct zone segment number was 3 to 11. So we arbitrarily divided the infarcts as small (6 affected segments or less) and large (>6 affected segments). Small and large infarcts were equally distributed among groups. There were 8 small and 8 large infarcts in the cell therapy group and 8 small and 8 large infarcts in the control group.

Echo left ventricular strain

A comprehensive two-dimensional transthoracic echocardiographic examination was performed using a Vivid E95 machine (GE Healthcare). Exams were performed by a single operator. Standard echocardiographic views were acquired, and ventricular and atrial chamber quantification was performed according to current recommendations ('Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging').[8] The images were optimized maintaining a frame rate between 50 and 70 fps, in order to ensure adequate quality in the subsequent analysis with speckle tracking. A post process analysis was made using a workstation ECHOPAC 101.1.2, and myocardial deformation was evaluated by the Q analysis application. Longitudinal strain of the apical four chambers, two chambers, and three views was assessed, and thus, longitudinal strain curves and the value of global longitudinal strain were obtained.

Follow-up

Patients had a basal ultrasound exam as well as a cardiac MRI. Follow-up controls at the external office were performed at 1, 3, and 6 months and 1 year after cell implantation. The ultrasound exam, cardiac MRI, and 24 h ECG were repeated 1 year after cell injection.

All 32 patients completed the follow-up period.

Patients excluded from the study

Three patients were excluded from the analysis because they did not reach the minimal pre-specified number of 1×10^6 CD133+ injected cells. In one additional patient, MRI measurements could not be performed due to image artefact distortion from an aortic vascular prosthesis. We analysed 32 randomized patients, allocated 16 to the cell therapy group and 16 to the control group.

Statistical analysis

The normality of distribution of continuous variables was tested by one-sample Kolmogorov–Smirnov test. Continuous variables with normal distribution were presented as mean (standard deviation); non-normal variables were reported as median (interquartile range). Means of two continuous normally distributed variables were compared by independent samples Student's *t*-test. Mann–Whitney *U* test was used to compare medians of two groups of variables not normally distributed. The frequencies of categorical variables were compared using χ^2 test. To assess the relation between continuous variables, we constructed linear regression plots. A value of *P* %3C 0.05 was considered significant.

We performed the following comparisons:

1 Total cell number injected was plotted against basal to 1 year increment in infarct zone volume and increment in left ventricular systolic strain. Infarct zone volume 1 year

Table 1 Basal features of the patients included (mean ± SD)

	Cell therapy $n = 16$	Control $n = 16$	P
Age (years)	63.5 ± 8.7	65 ± 8.2	n.s.
Male sex	93%	75%	n.s.
Cardiovascular risk factors			
Hypertension	56%	31%	n.s.
Smokers	81%	43%	n.s.
Diabetes	6%	19%	n.s.
Cholesterol	44%	50%	n.s.
MRI			
Ejection fraction (%)	43.9 ± 8.5	41.9 ± 12.2	n.s.
Infarct volume (mL)	21.1 ± 9.1	20.3 ± 12.4	n.s.
Infarct size (%)	15.7 ± 6.6	13.7 ± 7.7	n.s.
Number of necrotic LV segments	105	104	n.s.
Infarct location			
Anterior	81%	56%	n.s.
Inferior	19%	38%	n.s.
Lateral	0%	6%	n.s.
Treadmill test			
Time (s)	627 ± 142	534 ± 133	n.s.
METS	12.7 ± 2.6	10.1 ± 2.8	n.s.
Max heart rate (%)	89.4 ± 0.9	87.8 ± 11.4	n.s.
Blood test			
Neutrophils	4.19 ± 0.99	4.44 ± 1.32	n.s.
Monocytes	0.61 ± 0.18	0.63 ± 0.21	n.s.
Lymphocytes	1.89 ± 0.59	2.29 ± 0.86	n.s.
Glycaemia	105.4 ± 21.9	106.9 ± 24.3	n.s.
Creatinine	1.02 ± 0.21	0.97 ± 0.28	n.s.
LDL-cholesterol	77.7 ± 37.1	85.6 ± 14.5	n.s.
Medication			
Beta-blockers	25%	25%	n.s.
ACE inhibitors	50%	31%	n.s.
ARA	19%	37%	n.s.
Nitrates	0%	0%	n.s.
Calcium blockers	25%	12%	n.s.
Diuretics	6%	25%	n.s.
Statins	94%	100%	n.s.
Aspirin	94%	100%	n.s.

ACE, angiotensin-converting enzyme; ARA, angiotensin receptor antagonist; LV, left ventricular; METS, metabolic equivalents of task; MRI, magnetic resonance imaging; n.s., not significant.

reduction was plotted against the 1 year increment in left ventricular systolic strain.

2 We compared basal and follow-up left ventricular volumes and infarct zone between the treated and control groups.

3 We measured MRI left ventricular wall diastole to systole increase in millimetre (systolic thickness increase) in every single left ventricular segment. Then we compared left systolic thickness increase before and after 1 year in infarcted and healthy segments in cell therapy and control groups.

4 We compared basal and 1 year systolic thickness increase in the borders and the core of the infarct area in the treated and control groups.

5 Finally, to compare core regeneration in small vs. large infarcts, we calculated the infarct core basal to 1 year increment in each patient. Then we compared the medians between small and large infarcts in the cell therapy and control groups separately.

Results

Patient baseline features

Groups were quite similar in cardiovascular risk factors, exercise capacity, white cell count, glycaemia, creatinine, LDL-cholesterol, medication, and infarct location and extension (*Table 1*).

Cell collection yield

The blood apheresis procedure was very well tolerated, and there were no complications in any patient. The mean volume of the apheresis product obtained was 63.6 ± 10.7 mL. The mean number of cells obtained in the blood aphaeresis product was 14 784 ± 3805 × 10^6 CD45+ cells (range 6837 to 22 440 × 10^6), 40.5 ± 23.7 × 10^6 CD34+ cells (range 5.0 to 92.9 × 10^6), and 23.7 ± 14.5 × 10^6 CD133+ cells (range 4.3 to 58.9 × 10^6). So only 0.27 ± 0.17% (range 0.02% to 0.65%) were CD34+ cells and 0.16 ± 0.09% (range 0.04% to 0.32%) were CD133+ cells in the total volume of the blood aphaeresis product. Cell yield was not related to age, total white cell count, nor to any other clinical or haematological variable.

Cell injection

The total number of cells injected is expressed in *Table 2*. A mean of 1890 ± 398 × 10^6 CD45+ cells, ranging from a minimum of 1224 × 10^6 to a maximum of 3191 × 10^6 total white cells, was administered. The infusions contained a mean of

Table 2 Total number of cells injected

Patient	CD45+ cells injected	CD34+ cells injected	CD34 + % of cells	CD133+ cells injected	CD133 + % of cells	Injected volume (mL)
1	3 191 500 000	2 557 921	0.08	3 205 721	0.10	13
2	1 599 659 200	11 469 859	0.72	5 597 960	0.35	21
3	1 224 000 000	5 986 800	0.49	1 570 800	0.13	6
4	1 817 480 000	2 500 108	0.14	3 873 951	0.21	7
5	1 886 760 000	4 024 626	0.21	1 261 177	0.07	12
6	1 784 000 000	3 174 290	0.18	3 363 763	0.19	8
7	1 695 000 000	4 917 222	0.29	2 930 974	0.17	6
8	1 887 300 000	5 659 576	0.30	5 143 378	0.27	9
9	2 100 000 000	6 171 262	0.29	1 644 848	0.08	7
10	1 982 500 000	6 141 311	0.31	2 087 177	0.11	13
11	1 881 600 000	8 084 686	0.43	6 047 244	0.32	7
12	1 806 000 000	7 858 407	0.44	4 381 983	0.24	7
13	1 792 000 000	5 614 513	0.31	4 077 431	0.23	7
14	1 817 200 000	3 661 971	0.20	2 074 114	0.11	7
15	2 008 000 000	2 137 086	0.11	1 714 768	0.09	10
16	1 772 800 000	11 608 459	0.65	5 342 837	0.30	8
Mean	1 890 362 450	5 723 008	0.32	3 394 883	0.19	9.3
SD	398 215 884	2 896 035	0.18	1 589 171	0.09	3.9

5.7 ± 2.9 × 10^6 CD34+ cells, ranging from 2.1 to 11.6 × 10^6, and a mean of 3.4 ± 1.6 × 10^6 CD133+ cells, ranging from 1.3 to 6.0 × 10^6 CD133+ cells.

The number of CD34+ and CD133+ cells (a measure of blood mononuclear cells regenerative potential) in total apheresis product was high, but the number of CD133+ cells injected was limited by the total mononuclear cell number to be injected in order to avoid possible embolic complications. So, in three patients, the minimal pre-specified number CD133+ cell number of 10^6 was not achieved because it was needed an extremely high number of total cells to reach that CD133+ cell number.

There were no complications during or after intracoronary cell injection. Coronary flow was TIMI III in all patients, and there were no troponin and CK increases after the procedure.

Ventricular remodelling

Pre-treatment and post-treatment ventricular volumes are shown in *Table 3*. There were no differences in basal end-diastolic volume (EDV) between the treatment and control groups, but the cell therapy patients showed a reduction in EDV after treatment that was not observed in the control group. End-systolic volume, stroke volume, ejection fraction, and infarct volume were not different between group and did not show changes after the follow-up period.

Echo measurements are also shown in *Table 3*, and they showed no changes in left ventricular volumes between groups and after treatment. There were no differences in global left ventricular strain. Infarct zone segmental strain showed a non-significant improvement in the cell therapy group and remained unchanged in the control group at follow-up.

Table 3 Magnetic resonance imaging and echo basal and 1 year follow-up left ventricular volumes, infarct volume, and left ventricular strain in the cell therapy and control groups [median (interquartile range)]

	Cell therapy n = 16	Control n = 16	P
MRI			
End-diastolic volume (mL)			
Basal	161.4 (126.9–184.5)	184.2 (145.3–204.8)	n.s.
1 year	142.8 (120.5–180.9)	167.6 (155.0–197.5)	%3C0.05
End-systolic volume (mL)			
Basal	90.3 (76.1–102.4)	100.9 (78.1–143.2)	n.s.
1 year	82.9 (68.3–100.3)	98.2 (82.6–121.0)	n.s.
Stroke volume (mL)			
Basal	66.8 (51.3–85.4)	71.9 (64.9–79.0)	n.s.
1 year	62.8 (46.0–76.0)	71.0 (62.9–87.8)	n.s.
Ejection fraction (%)			
Basal	45.4 (40.5–50.8)	43.1 (34.1–46.8)	n.s.
1 year	45.2 (39.9–49.4)	42.1 (36.0–49.4)	n.s.
Infarct volume			
Basal	21.1 (13.6–26.6)	15.6 (13.3–24.2)	n.s.
1 year	22.9 (14.4–30.4)	18.6 (12.4–21.8)	n.s.
Echo			
End-diastolic volume (mL)			
Basal	114 (100.5–129)	141 (122.5–172.5)	n.s.
1 year	114 (103.5–125)	129 (116.5–176)	n.s.
End-systolic volume (mL)			
Basal	53 (47.5–65.5)	69 (60.5–93)	n.s.
1 year	54 (43.5–65)	651 (57–95)	n.s.
Ejection fraction (%)			
Basal	51 (45.5–56)	48 (43–51)	n.s.
1 year	53 (49.5–56)	52 (42–56)	n.s.
Global left ventricular systolic strain			
Basal	−14.2 (−15.8 to −12)	−11 (−15.9 to 10.3)	n.s.
1 year	−15 (16.6 to −11.2)	−11.8 (−17 to −10.2)	n.s.
Scar zone segmental systolic strain			
Basal	−5.8 (−10.6 to −2.1)	−3.2 (−10.2 to −1.9)	n.s.
1 year	−8.2 (−11.9 to −2.6)	−3 (−15.4 to −1.3)	n.s.

MRI, magnetic resonance imaging; n.s., not significant.

Cell number to reduce infarct volume

Infarct volume reduction was related to the amount of CD133 + and CD34+ cells injected. CD133+ cell number relation to scar volume reduction and segmental strain improvement is shown in *Figure 1A* and *1C*. Infarct volume reduction was related to segmental strain improvement (*Figure 1B*). CD34+ cell number was related to infarct volume reduction (R = 0.63; P %3C 0.01). Total white cell (CD45+) number injection was not related to infarct volume reduction nor to improvements in infarct zone segmental strain.

Total left ventricular segment analysis

When all (healthy + necrotic) ventricular segments were analysed, we observed trend to an improvement in MRI systolic thickness increase in cell therapy group 1 year after treatment [1.71 (0.38–3.56) vs. 2.00 (0.70–3.42) mm; P = not significant (n.s.)]. Control patients showed a non-significant deterioration in systolic thickness increase at 1 year follow-up [2.02 (0.75 to 3.4) vs. 1.91 (0.77–3.17) mm; P = n.s.] (*Figure 2*).

Infarct zone function recovery

Magnetic resonance imaging infarct segments systolic thickness improved after treatment [0.42 (−0.38 to 1.14) vs. 1.06 (−0.10 to 2.12) mm; P %3C 0.01] in the cell therapy group and showed a non-significant deterioration in the control patients [1.05 (0.21–1.77) vs. 0.81 (−0.06 to 1.64) mm; P = n.s.] (*Figure 2*).

Infarct borders and core function recovery

Cell therapy

Only the borders of the infarct zone improved after treatment, while the core showed no improvement in its systolic

FIGURE 1 Infarct volume reduction relation to the number of CD133+ cells injected (R = 0.53; P %3C 0.05) (A). Infarct volume reduction relation to infarct zone segmental strain (R = 0.64; P %3C 0.01) (B). Number of CD133+ cells injected relation to necrotic zone segmental strain (R = 0.616; P % 3C 0.01) (C).

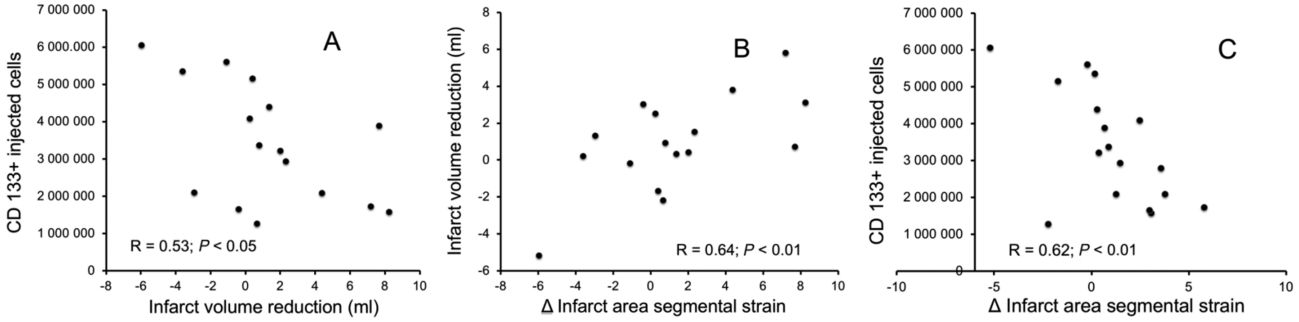
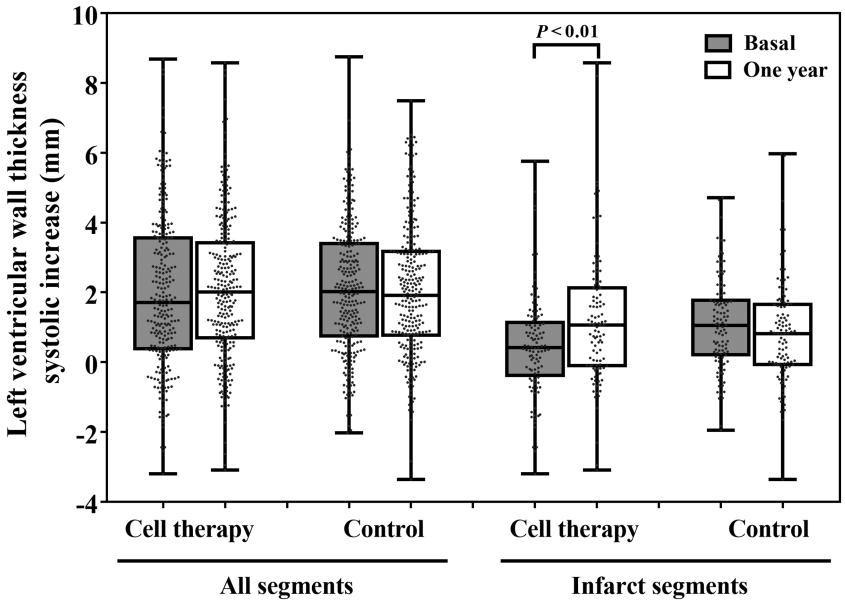


FIGURE 2 Left ventricular systolic thickness increase in all segments (left) in the cell therapy group [basal: 1.71 (0.38–3.56), 1 year: 2.00 (0.70–3.42) mm; P = n.s.] and in the control patients [basal: 2.02 (0.75 to 3.4), 1 year: 1.91 (0.77–3.17) mm; P = n.s]. Left ventricular systolic thickness increase at the infarct area (right) in the cell therapy group [basal: 0.42 (−0.38 to 1.14), 1 year: 1.06 (−0.10 to 2.12) mm; P %3C 0.01] and in the control patients [basal: 1.05 (0.21–1.77), 1 year: 0.81 (−0.06 to 1.64) mm; P = n.s]. Data are expressed as median (interquartile range).

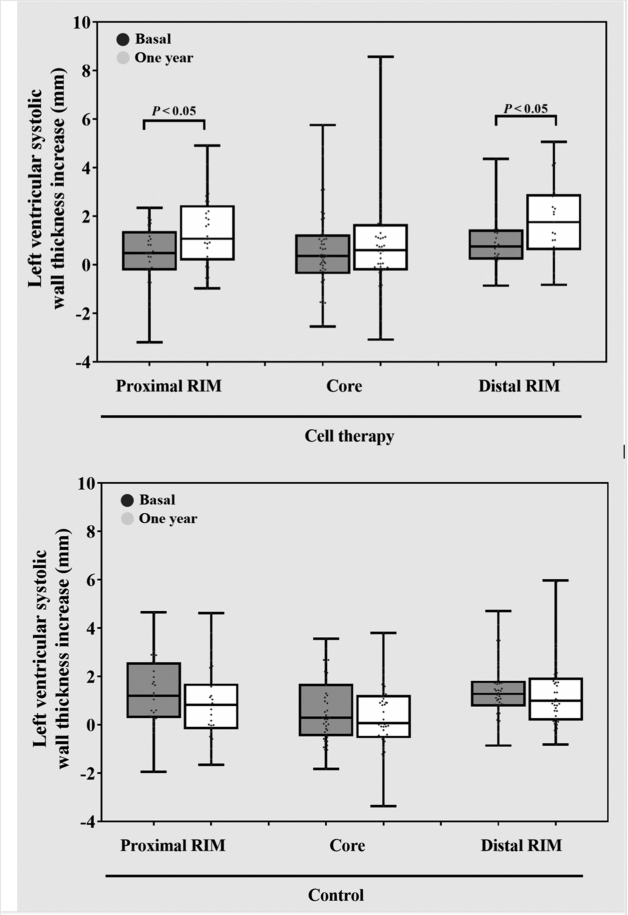


thickness 1 year after cell infusion [proximal rim: 0.48 (−0.18 to 1.33) vs. 1.07 (0.22–2.40) mm; P %3C 0.05, distal rim: 0.75 (0.26–1.40) vs. 1.76 (0.65–2.86) mm; P %3C 0.05, core: 0.36 (−0.33 to 1.20) vs. 0.60 (−0.18 to 1.62) mm; P = n.s.]. Thus, only the core of small infarct areas showed a clear recovery after treatment [infarcts with 6 affected segments or less vs. more than 6 segments systolic thickness post-treatment increment: 0.28 (−0.19 to 0.71) vs. −1.21 (−2.60 to −0.53) mm; P %3C 0.01] (*Figure 3*).

Control patients

There were no significant changes in the rim or the core of the infarct zone at follow-up in the control group. There was a non-significant deterioration of systolic thickness increase in both the rim and the infarct core [proximal rim: 1.20 (0.33–2.53) vs. 0.82 (−0.13 to 1.65) mm; P = n.s., distal rim: 1.24 (0.80–1.72) vs. 0.96 (0.19–1.81) mm; P = n.s., core: 0.30 (−0.42 to 1.64) vs. 0.07 (−0.60 to 1.20) mm; P = n.s.] (*Figure 3*).

FIGURE 3 Left ventricular systolic thickness increase at the borders and the scar core before and 1 year after cell injection. (top) Cell therapy group: proximal rim [basal: 0.48 (−0.18 to 1.33), 1 year: 1.07 (0.22–2.40) mm; *P* %3C 0.05], core [basal: 0.36 (−0.33 to 1.20), 1 year: 0.60 (−0.18 to 1.62) mm; *P* = n.s.], and distal rim [basal: 0.75 (0.26–1.40), 1 year: 1.76 (0.65–2.86) mm; *P* %3C 0.05]. (bottom) Control group: proximal rim [basal: 1.20 (0.33–2.53), 1 year: 0.82 (−0.13 to 1.65) mm; *P* = n.s], core [basal: 0.30 (−0.42 to 1.64), 1 year: 0.07 (−0.60 to 1.20) mm; *P* = n.s.], and distal rim [basal: 1.24 (0.80–1.72), 1 year: 0.96 (0.19–1.81) mm; *P* = n.s]. Data are expressed as median (interquartile range).



The core of small infarct areas in the control group showed no recovery at all at 1 year follow-up [infarcts with 6 affected segments or less vs. more than 6 segments systolic thickness 1 year increment: −0.84 (−1.12 to 0.44) vs. −0.45 (−1.11 to 0.21) mm; *P* = n.s.].

Safety measures

There were no adverse cardiac events during the study period. No other adverse side side-effects were registered in any patient. Twenty-four ECG recordings were performed before and at follow-up. There were no rhythm disturbances recorded after cell therapy or in the control group.

Discussion

Patient selection

We designed a protocol to include patients with ischaemic heart disease, who had suffered a myocardial infarction episode more than 3 years earlier, treated with primary angioplasty, with permeable culprit coronary arteries and TIMI III flow, in stable conditions, and no heart failure signs. Acute myocardial infarction is followed by a 2 to 3 year time interval in which left ventricular remodelling takes place and coronary thrombotic complications are more common. We sought to avoid such confounding variables and study patients in stable conditions. Timely primary angioplasty provides a better reperfusion method compared with thrombolysis and offers a better opportunity to maintain a basic muscle and microvessel structure at the infarct zone, providing a frame for regenerative cell arrival and tissue healing.

Patient baseline features are those of the classical patient with an old myocardial infarct in the primary angioplasty era. They are shown in *Table 1*. Age, sex, and risk factors were well distributed among groups. Functional variables relevant to the purpose of the study, such as mild depressed ejection fraction, anterior/inferior infarct location ratio, and good treadmill test duration and metabolic equivalents of task achieved, were suited for purpose of the study and similar in both groups. There were no differences in white cell count, glycaemia, renal function, or LDL-cholesterol, which could have affected the results, and they all received standard post-myocardial infarction medication.

The clinical frame common to both groups is that of a patient with stable ischaemic heart disease, moderate size scar, mild depressed left ventricular ejection fraction, and intact functional capacity. These patients are the most frequent group in the primary angioplasty era. Atherothrombotic risk is well controlled in these patients with statin and antithrombotic therapy, but they are at risk of developing left ventricular dilatation and, ultimately, heart failure. These patients are probably the population in which this therapy should be performed.

Apheresis extraction protocol and cell product

We performed blood apheresis in 20 patients with an old myocardial infarction aged 55 to 77 years. Blood apheresis is a minimally invasive procedure that was well tolerated by all patients. There were no peri-procedural complications. Autologous bone marrow mononuclear cells were used in most trials. Harvesting bone marrow autologous cells in sick patients is not always feasible due to the patient's clinical condition, concomitant antithrombotic treatment, and other logistical and economic limitations. Even clinical trials in stable patients are difficult to complete because of limited

patient recruitment due to aggressive cell collection techniques and patient clinical conditions, which, in addition, exclude severely ill patients who may benefit most from cell-based therapy.

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 even by very sick patients, it requires no cell manipulation, and the aphaeresis product is infused shortly after cell collection, thus avoiding cell viability deterioration and death and potential complications.

Patient age and co-morbidities are not a limitation to the utilization of mobilized mononuclear cells. Cell mobilization is reduced by a 25% in patients >55 years, but not cell engraftment.[9] Our patients were all over 55 years of age, and although co-morbidities could further reduce cell collection yield, our data show a high total CD34+ and CD133+ cell count in the aphaeresis product, which gives plenty of room to increase cell injection number in future studies.

Finally, the aphaeresis product is a combination of a wide range of white cells. The ability of each cell type to promote tissue regeneration (i.e. blood precursor cells, mesenchymal cells, and resident stem cells) is related to the particular molecular environment these cells are in contact with. In the REGENERATE randomized trial, the group that received G-CSF + intracoronary mononuclear cells showed the best results in terms of the recovery of the infarct zone contractile function.[4] Also, infiltrating neutrophils activate monocyte-derived macrophages to induce new vessel formation and inflammatory debris clearance in a myocardial infarction rat model.[10] Thus, it might well be that the cell cocktail that accompanies CD34+ and CD133+ regenerative cells in the aphaeresis product has a role in tissue recovery.

Total number of cells injected

Cells selected for this experiment included CD34+ and CD133 + cells, which have angiogenic and regenerative capacities. Because the percentages of CD34+ and CD133+ cells were low in the aphaeresis product (0.27% and 0.16%) and that cell number is important to infarct recovery, we increased the maximal number of cells injected to 3000 × 10^6, which is higher than in any published study. Still, there were three patients in whom the total cell number would have been too high to inject >10^6 CD133+ cells and were excluded from analysis. This means that 15% of patients could not benefit from this particular cell collecting technique unless we substantially increase total cell number to >5 × 10^9. In any case, we went one step further, and this is the first study to inject >1 × 10^9 mononuclear cells in the coronary vessel responsible for a chronic myocardial infarction. We injected a mean of 1890 ± 398 × 10^6 CD45+ and 5.7 ± 2.9 × 10^6 CD34+ cells. As a reference point, in the randomized REGENERATE trial, a mean of 216 ± 221 × 10^6 mononuclear cells and 4.9 ± 2.7 × 10^6 CD34+ cells was injected.[4] In addition, we collected these cells not by invasive bone marrow puncture but by blood apheresis.

Animal and human studies show a clear relationship between the number of CD34+ cells injected and ventricular function recovery and infarct size reduction.[6,11] We found a linear relationship between the number of CD34+ and CD133+ cells injected and the reduction of infarct volume (*Figure 1A*). So it is of maximal importance to obtain and inject a high number of precursor cells to achieve a significant recovery of the infarct zone. As seen earlier, the number of CD34+ and CD133+ total cells collected in the apheresis product is high [40.5 ± 23.7 × 10^6 CD34+ (range 5.0 to 92.9 × 10^6) and 23.7 ± 14.5 × 10^6 CD133+ cells (range 4.8 to 57.9 × 10^6)] and allows to increase cell number and regenerative capacity in future studies.

Left ventricular remodelling

Our results show that cell therapy could prevent ventricular remodelling. There were no differences in left ventricular volumes between the two groups before treatment, but EDV was significantly smaller in the cell therapy group at follow-up (*Table 3*). This reduction in ventricular volumes could be driven by the improvement in MRI systolic thickness at the infarct zone and preservation of systolic thickness in all ventricular segments after therapy, while the deterioration of systolic thickness of the infarct zone in the control group could ballast the contractility of the healthy segments observed at follow-up. Finally, scar zone contractility improvement will help maintain contractility in the nearby healthy segments and help to reduce ventricular volumes after cell therapy. The opposite effect is seen in non-treated patients, in whom scar contractility deterioration decreases healthy segments contractility and leads to left ventricular dilatation to maintain an adequate stroke volume.

Infarct area regeneration

The beneficial effect of cell therapy was observed at high cell number injection. The number of cells injected, infarct volume reduction, and improvement in infarct zone contractility were interrelated (*Figure 1A–1C*). So, to achieve a significant regeneration of the infarct area, high cell number injections, much higher than the ones employed until now, are needed. Scar zone systolic myocardial thickness significantly increased after treatment in the cell therapy group while showed a non-significant deterioration in the controls (*Figure 2*). The functional improvement produced by cell injection could only be related to a regeneration of the infarcted area.

The segments located proximal and distal to the infarct zone recovered contractile function to figures close that observed in the non-infarcted segments in the treated group, while the central core of the scar tissue did not improve. All the scar segments of the control group had a non-significant contractile deterioration (*Figure 3*).

Precursor cell probably acts by a paracrine mechanism, increasing viable myocardium by inducing the proliferation of resident myocytes at the borders of the scar zone. These are mosaic areas with a mixture of interspaced myocytes and fibrosis, but with a dense capillary network that allows the arrival of injected cells and neo-vessel proliferation as well as myocyte recolonization. As a result, small infarcts showed an almost complete recovery of contractile function while large ones not. Based on these data, it seems that the scar zone reduces its size from the outer rim to the core. So small infarct areas recover better than large ones. Future approaches should include intracoronary delivery with higher cell count aiming to advance the regenerative border to the nucleus of the scar core.

We did not obtain a reduction in MRI infarct zone volume in all patients, although there was a clear improvement in infarct zone contractility. These contradictory results could be due to the lack of ability of MRI to detect mosaic areas in which there is fibrosis (well detected by MRI) and myocardial regeneration (which improves contractile function). A myocardial perfusion scan would answer that question and should be performed at least in a patient subgroup in studies to be performed.

Impact of cell delivery technique on scar regeneration

The TACTICS (Transnational Alliance for Regenerative Therapies in Cardiovascular Syndromes) Group global position paper on cardiovascular regenerative medicine group[12] ideal cell therapy delivery technique: safe, user-friendly, clinically proven, and inexpensive is far from being found. Surgical direct intramyocardial injection is limited to open-chest surgery patients. Intravenous administration has shown no effect. So it seems that percutaneous intracoronary or intramyocardial techniques are the best placed. Intramyocardial cell injection can be performed percutaneously and has the advantage of reaching the centre of the scar zone by injecting cell at the precise scar locations to be regenerated. But it carries the risk of creating separate cell clusters that could produce reentrant electrical circuits and potentially induce ventricular arrhythmias.[13] We have chosen intracoronary cell injection via radial artery catheterization. It is easily performed and has a negligible complication rate.[5,12] Intracoronary cell injection placing a catheter at the former culprit lesion assures cell delivery right to the target zone.

Limitations of the study and future directions

The present research work limitations are that cell selection technique could be considered vintage and cell delivery method does not reach the complete extension of the scar zone. But we think we have opened the door to future experiments. We have chosen a safe and simple technique to collect cells with regenerative capacity. This technique is also inexpensive, available to most medical centres, can be performed in a large number of patients, and is safe and carries no adverse effects. Intracoronary cell injection is also a safe and inexpensive method to reach the scar zone.

So future directions to improve/analyse scar regeneration include the following:

1. Select patients appropriately: candidates should have had a primary angioplasty procedure and have TIMI III flow at the present time.
2. Increase the number of cells injected. Because the intracoronary injection of higher numbers of cells has not shown embolic or inflammatory complications, future studies should try to inject up to 5×10^9 total mononuclear cells, which will allow to inject $>7 \times 10^6$ CD133+ cells. The apheresis product has enough absolute number of CD133+ cells to warrant this protocol.
3. Reinjection is a possibility. Intracoronary injection offers an inexpensive and safe method of cell injection and scar regeneration.
4. Myocardial perfusion scans will better assess scar regeneration.

Conflict of interest

None declared.

Funding

This research work was funded by Grupo Quiron Salud Hospitals, Spain.

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