

Recovery of regional but not global contractile function by the direct intramyocardial autologous bone marrow transplantation - Results from a randomized controlled clinical trial

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**RECOVERY OF REGIONAL BUT NOT GLOBAL CONTRACTILE FUNCTION BY  
DIRECT INTRAMYOCARDIAL AUTOLOGOUS BONE-MARROW  
TRANSPLANTATION. RESULTS FROM A RANDOMIZED CONTROLLED  
CLINICAL TRIAL.**

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**Short title : Bone-marrow delivery in myocardial infarction**

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## ABSTRACT

### Introduction

Recent trials have shown that intracoronary infusion of bone marrow cells (BMC) improves functional recovery after acute myocardial infarction. However, whether this treatment is effective in heart failure as a consequence of remodelling after organized infarcts remains unclear.

### Hypothesis

In this randomized trial, we assessed the hypothesis that direct intramyocardial injection of autologous mononuclear bone marrow cells during CABG could improve global and regional left ventricular ejection fraction (LVEF) at 4 months follow-up.

### Methods

Twenty patients (age  $64.8 \pm 8.7$ ; 17 male/3 female) with a postinfarction nonviable scar, as assessed by Thallium (Tl) scintigraphy and cardiac MRI, scheduled for elective CABG, were included. They were randomized to a control group ( $n=10$ , CABG only), or a BMC group (CABG and injection of  $60.10^6 \pm 31.10^6$  BMC). Primary endpoints were global LVEF change and wall thickening changes in the infarct area from baseline to 4 months follow-up, as measured by MRI. Changes in metabolic activity were measured by Tl scintigraphy and expressed as a score

with a range from 0-4, corresponding to % of maximal myocardial Tl uptake (4<50%, non-viable scar, 3=50-60%, 2=60-70%, 1=70-80%, 0>80%).

## Results

Global LVEF at baseline was  $39.5 \pm 5.5\%$  in controls and  $42.9 \pm 10.3\%$  in the BMC group ( $p=0.38$ ). At 4 months, LVEF had increased to  $43.1 \pm 10.9\%$  in the control group and to  $48.9 \pm 9.5\%$  in the BMC group ( $p = 0.23$ ). Systolic thickening had improved from  $-0.6 \pm 1.3$  mm at baseline to  $1.8 \pm 2.6$  mm at 4 months in the cell-implanted scars, whereas non-treated scars remained largely akinetic ( $-0.5 \pm 2.0$  mm at baseline compared to  $0.4 \pm 1.7$  mm at 4 months,  $p=0.007$  control vs BMC treated group at 4 months). Defect score decreased from 4 to  $3.3 \pm 0.9$  in the BMC group and to  $3.7 \pm 0.4$  in the control group ( $p = 0.18$ )

## Conclusions

At four months, there was no significant difference in global LVEF between both groups, but a recovery of regional contractile function in previously non-viable scar was observed in the BMC group.

**Key Words:** Stem cells, myocardial infarction, clinical trial

## **INTRODUCTION**

Experimental studies have shown that transplantation of bone-marrow derived cells may contribute to the regeneration of infarcted myocardium.<sup>1</sup> Based on those data, two clinical trials have shown that intracoronary infusion of autologous bone-marrow cells improves left ventricular functional recovery after acute myocardial infarction and successful percutaneous coronary intervention.<sup>2,3</sup> However, whether this treatment is effective in congestive heart failure as a consequence of remodelling after organized infarcts, remains unclear. Loss of viable myocardium initiates a process of adverse remodelling, leading to chamber dilatation and contractile dysfunction. Also, the efficiency of cell transfer in case of intracoronary administration may be hampered by the fact that only a small number of cells remain in the heart.<sup>4</sup> Therefore, we carried out a randomized controlled trial to assess the functional effect of direct intramyocardial injection of autologous bone-marrow cells on left ventricular ejection fraction (LVEF) in patients with a chronic myocardial infarction, undergoing coronary artery bypass graft (CABG) surgery.

## **METHODS**

### **Patient selection**

Patients were eligible if they were admitted for elective CABG surgery, had a transmural myocardial infarction on ECG and akinesia or dyskinesia in part of the left ventricle as shown by angiography. Patients needing urgent surgery were excluded, as well as patients in advanced

renal or hepatic failure or with documented malignancy. In addition patients carrying a pacemaker were excluded. The randomized controlled trial was approved by the local Ethics Committee. All patients provided written informed consent.

#### Randomisation and baseline investigation

Patients were randomly allocated in a 1:1 ratio to either the control, conventional CABG or bone-marrow-cell (BMC) group, by means of sequentially numbered, sealed envelopes. After randomisation, all patients underwent baseline investigations prior to revascularisation, consisting of cardiac MRI and thallium scintigraphy. Mean time between occurrence of infarct and CABG was  $217 \pm 162$  days in the BMC group and  $213 \pm 145$  days in the control group ( $p=0.95$ ).

#### Harvest and transfer of bone-marrow cells

In the bone-marrow-transplant group ( $n=10$ ), 40 ml of bone marrow was aspirated under local anaesthesia from the patients' iliac crest, the day before surgery. In the control group ( $n=10$ ), bone marrow (7 ml) was harvested from patients' sternum under full anaesthesia just before surgery, to ascertain that characteristics of the BMC suspension did not significantly differ between both groups. Bone marrow-derived mononuclear cells were immediately isolated by density gradient centrifugation using Lymphoprep (Nycomed). Isolated cells were washed twice with saline and subsequently resuspended in X-Vivo 15 medium (Cambrex) supplemented with 2% autologous serum. This cell suspension was transferred to Teflon Bags (Vuelife CellGenix) at a concentration of approximately  $1 \times 10^6$  cells/ml for overnight cultivation. The next day, cells were harvested and washed 3 times before finally suspended in 10 ml heparinized saline. Just

before transplantation, cells were filtered by a 70-micron cell strainer (Falcon). Nucleated cell viability was assessed by Trypan blue (Invitrogen) exclusion.

The complete 10 ml was directly injected at the border zone of the infarct scar. This was accomplished using 29 Gauge myojector syringes (Terumo) containing 0.5 ml fractions of the cell suspensions. Multiple punctures were carried out with a pre-bent needle so as to make injections parallel to the epicardium and avoid delivery of cells into the ventricular cavity. In the control group, an equivalent volume of heparinized saline was injected. The surgeon was unaware whether cells or only saline was injected. Revascularisation of the infarct related area was systematically carried out.

Complete revascularisation was achieved in all patients.

#### Characterisation of transplanted cells

The absolute counts of CD34 positive cells were determined using the stem-kit CD34+HPC Enumeration kit (Beckman Coulter).

As quality ex-vivo control, haematopoietic colony-forming cell growth was calculated by standard methylcellulose assay as described previously.

Further characterization of the transplanted cells was performed on a FACS Aria (Beckton & Dickenson) with 4-colour flowcytometry.

#### Follow-up

Prior to hospital discharge (9-14 d post surgery) and again four months after surgery, cardiac MRI and thallium scintigraphy were repeated in all patients.

To assess whether intramyocardial BMC transfer was associated with proarrhythmic effects, all patients who accepted underwent programmed ventricular stimulation at 6 to 8 weeks follow-up. Stimulation was done at the right-ventricular apex and the right-ventricular outflow tract with single, double and triple extra stimuli at twice the diastolic threshold and basic cycle lengths of 500 and 400 ms.

#### Cardiac MRI

Cardiac MR was carried out in a 1.0 T scanner (Gyrosan T 10-NT Philips Medical Systems) using surface body coil with ECG gating and respiratory triggering.

Sequence parameters were as follows:

- Balanced FFE gradient echo sequence with dynamic sequences in transversal plane, long axis, short axis and 4-chamber view.
- 3 D T1 weighted FFE gradient echo sequence with multishot and TFE prepulse with variable delay between 200 and 300 ms after IV contrast injection of megluminegadoterate (Guerbet, Roissy, France) ('delayed enhancement').

The balanced FFE gradient echo images were analysed in cine view to detect dyskinetic or akinetic regions and wall thickness measurement was done on short axis and long axis views.

Contrast enhanced MRI was used to assess myocardial injury after infarction and to differentiate between necrosis and viable tissue with scanning after a delay time of 15 min after IV injection of 15 ml 0.5 mmol/kg megluminegadoterate (delayed enhancement).



All images were analyzed by one investigator, unaware of treatment assignment (EB). Endocardial and epicardial borders were traced in all end-diastolic and end-systolic short axis and long axis slices to determine left ventricular end-diastolic volume (LVEDV) and end-systolic volume (LVESV) for calculation of global left-ventricular ejection fraction, with ejection fraction  $= ((\text{LVEDV} - \text{LVESV}) / \text{LVEDV}) * 100\%$ .

Regional wall thickening was calculated by determining the short axis slice through the center of the infarct region. A 19-segment model was used and pathologic segments were identified as segments with marked decrease in wall thickness and without thickening during systole. Correlation was made with pathologic regions on delayed enhancement. Wall thickening was calculated by the formula end-systolic thickness minus end-diastolic thickness.

Area length ejection fraction of the left ventricle was calculated midventricular on long axis views.

Correlation was made with thallium scintigraphy for evaluation of areas of myocardial scar.

### Thallium scintigraphy

Thallium scintigraphy was performed 10 min and 4 hours after the IV injection of 111 MBq  $^{201}\text{Tl}$  (Tyco Healthcare). Patients were injected at rest, after an overnight fasting period. All studies consisted of SPECT acquisition with a dual-head gamma camera (GE Hawkeye). Images were acquired over a 180° arc with camera heads in rectangular position (60 steps, 50 beats/step, matrix size  $64^2$  zoom 1.33). Image reconstruction was carried out with filtered back projection (Butterworth filter: cut-off 0.4-0.65 cycl/cm, order 5) followed by the reorientation of the

tomographic data. Short axis, vertical and horizontal long axis slices of the myocardium were generated on which a 20% background subtraction was done and a 16 step colour scale was applied, yielding a fixed image set used for further analysis.

Each study was evaluated by the consensus of 2 investigators, blinded for treatment assignment.

In order to obtain a semi-quantitative measurement of TI-uptake, the myocardium was divided into 17 segments, each receiving a score with a range from 0-4, corresponding to percentage of maximal myocardial TI uptake (4 < 50%, non-viable scar; 3=50-60%; 2=60-70%; 1=70-80%; 0>80%). The overall decrease in TI-uptake was then expressed as the sum score, obtained by adding up all segmental values.

### Statistical analysis

Continuous variables are reported as mean  $\pm$  SD. The LVEF and volume indices of the two groups were statistically compared by means of the Student's t test. The analyses were performed for the different measurement occasions and for the changes versus baseline. The non-parametric Wilcoxon-rank test was also used as an alternative test since the data are likely not normally distributed and the sample size of the study is small. ANCOVA was used to compare the changes in LVEF and volume indices in the two groups, with treatment as the main factor and baseline EF or volume index as a covariate. For each patient the wall thickening was measured in a number of ventricular segments. These measurements are not independent observations. Therefore mixed model methodology was used to investigate the changes in wall thickening between groups and alternatively Friedman tests to check the differences with a non-parametric test, taking into account that measurements in ventricular segments are not independent observations. Categorical variables were compared using the  $\chi^2$ -test and Fishers Exact test. Statistical

significance was assumed at a value of  $p < 0.05$ . Computations were performed with SAS 9.1 (SAS Institute, Inc., Cary, NC).

#### Statement of Responsibility

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

## RESULTS

Between January 13<sup>th</sup> 2004 and December 22<sup>nd</sup> 2004, 28 patients were assessed for eligibility. Four patients did not meet the inclusion criteria, based on preoperative MRI and Thallium. One patient refused to participate. Twenty-three patients were randomized, 11 to the bone marrow transplant group and 12 to the control group. In the control group one patient died on the fifth postoperative day due to multi-organ failure secondary to low cardiac output syndrome. One patient in the control group was lost to follow-up due to acute psychiatric illness. In the bone-marrow group, one patient died on postoperative day 7 due to a cardiac unrelated cause (perforated oesophageal ulcer complicated by mediastinitis). The final cohort included 10 controls and 10 patients in the bone-marrow-cell group. Table 1 shows baseline characteristics of the treated groups.

The total amount of recovered BMCs used for transplantation was on average  $60.25 \times 10^6 \pm 31.35 \times 10^6$  nucleated cells (viability  $95.05\% \pm 2.54\%$ ; recovery  $73.0 \pm 14.6\%$ ). This transplanted cell suspension contained  $1.42\% \pm 0.99\%$  CD34+ cells and  $76.37 \pm 44.47$  CFU-GM

per  $10^5$  mononuclear cells. More detailed information about the characteristics of the BMC suspension is described in Table 2.

Global ejection fraction increased in both groups, albeit not significantly. In the control group, it was  $39.5 \pm 5.5\%$  at baseline,  $41.2 \pm 10.1\%$  at hospital discharge and  $43.1 \pm 10.9\%$  at 4 months ( $p=0.36$  baseline vs 4 months). In the BMC group LVEF was  $42.9 \pm 10.3\%$  at baseline. Baseline values were not significantly different between groups ( $p = 0.19$ ). LVEF increased to  $45.8 \pm 13.2\%$  at discharge and  $48.9 \pm 9.5\%$  at 4 months ( $p=0.21$  baseline vs 4 months). There was no significant difference in improvement between the control and the BMC treated group at 4 months follow-up ( $p = 0.41$ ).

Changes in LVEDV index and LVESV index did not differ significantly between the control and BMC groups (Table 3).

In all patients, thallium scintigraphy was performed prior to surgery, and repeated at hospital discharge and at 4-months follow-up. In the final analysis, only segments in the area with intention to treat with a Tl-uptake score of 4 at 10 min and 4 hours were considered. In the BMC group, defect score decreased to  $3.5 \pm 0.9$  at discharge and further to  $3.3 \pm 1.0$  at 4 months. In the control group, defect score was  $3.7 \pm 0.4$  at hospital discharge, representing the revascularisation effect on Tl-uptake. No further decrease in defect score was observed at 4 months ( $3.7 \pm 0.4$ ) (Fig 1). The differences were not statistically significant at hospital discharge ( $p=0.51$ ), nor at 4 months follow-up ( $p=0.63$ ).

According to MRI, 35 pathologic segments were identified in the BMC group and 39 in the control group. The presence of an infarct was confirmed by thallium uptake less than 50% in that area. Wall thickening in the affected segments was  $-0.5 \pm 2.0$  mm at baseline in the control group and  $-0.6 \pm 1.3$  mm in the BMC group. At hospital discharge wall thickening values did not differ significantly from baseline in both groups:  $0.4 \pm 1.6$  mm in the control group and  $0.3 \pm 1.6$  mm in

the treatment group ( $p=0.18$ ). At 4 months however, a significant improvement in wall thickening was observed in the BMC group ( $1.8\pm 2.6\text{mm}$ ), whereas in the control group, wall thickening remained unchanged ( $0.4\pm 1.7\text{mm}$ ) ( $p = 0.007$ ; Fig. 2).

The BMC group could be divided into a subgroup of patients showing a response ( $n=5$ ), a group of non-responders ( $n=4$ ) and one patient with discordant findings. The subgroup of responders was defined as those patients, showing a significant wall thickening at 4 months postoperatively and decrease in thallium defect score. In this subset of patients, wall thickening increased from  $-0.6\pm 1.3\text{ mm}$  to  $3.0\pm 2.6\text{ mm}$ . Thallium defect score decreased from 4 to  $2.6\pm 1.0$ . In the group of non-responders, no wall thickening was observed at 4 months follow-up (from  $-0.7\pm 1.5\text{ mm}$  preoperatively to  $0.0\pm 1.6\text{ mm}$  at 4 months follow-up) and defect score remained 4 in all segments. In the patient with discordant findings, improvement in regional contraction was observed, whereas no change in thallium uptake defect score was noted.

The improvement in the responder group did not correlate with the number of transplanted mononuclear cells ( $63.8\times 10^6\pm 41.6\times 10^6$  cells in the responder group vs  $57.9\times 10^6\pm 30.3\times 10^6$  cells in the non-responder group,  $p=0.82$ ). Interestingly, the number of CD34+ cells in the responder group was significantly higher than in the non-responder group ( $3.1\pm 1.97\%$  vs  $0.9\pm 0.38\%$ ,  $p=0.03$ ). Table 4 gives an overview of the total number of transplanted cells, the percentage and absolute number of CD34 positive cells and the TI defect score and wall thickening changes for each patient in the BMC group. Also, the greater improvement in global LVEF (4M versus baseline) was observed in patients with higher numbers of engrafted CD34 positive cells. Fig 3 shows LVEF changes (4M-baseline) for each BMC patient, plotted as a function of absolute numbers of CD34+ cells implanted ( $r=0.49$ ).

Additional investigations suggest that direct intramyocardial injection of cells does not cause additional damage to the myocardium. Although 1 patient died in the treated group, the cause of

death was non-cardiac related. Peak CK-total and CK-MB values were similar in both groups (CK-total  $1308 \pm 689$  vs  $824 \pm 209$  U/L in BMC vs control group,  $p=0.09$ ; CK-MB  $48 \pm 44$  vs  $40 \pm 29$  U/L,  $p=0.73$ ). Also peak CTnI values did not differ significantly between both groups ( $6.6 \pm 4.1$  U/L vs  $9.7 \pm 12.8$  U/L BMC versus control group,  $p=0.68$ ).

Five control patients and 9 patients who received bone marrow transfer agreed to undergo an electrophysiological study. In the BMC group, monomorphic ventricular tachycardia (VT) could be induced in 5 patients, polymorphic VT in 1. In 3 out of those 6 patients, an AICD was implanted. Three patients were treated with amiodarone and closely followed-up. No patient in the control group had inducible VT.

To investigate a possible confounding effect of amiodarone on the BMC group data, a subgroup analysis was carried out. Wall thickening change (4M-baseline) in the amiodarone-treated group was  $2.0 \pm 3.0$  mm versus  $3.0 \pm 2.1$  mm in the non-amiodarone-treated patients ( $p=0.24$ ) indicating that amiodarone had no significant effect on cell treatment.

## DISCUSSION

This randomized clinical trial addresses the effect of autologous BMC therapy on LVEF in patients with ischemic cardiomyopathy as a consequence of postinfarction myocardial scar. Patient characteristics in both groups were comparable. Complete revascularisation was achieved in all patients, but a marginally higher number of bypass grafts were constructed in the BMC group ( $2.8 \pm 0.4$ ) compared to the control group ( $2.5 \pm 0.5$ ,  $p=0.07$ ). Although these grafts were placed in remote, non-infarcted areas, they might have increased perfusion in the neighboring scar tissue and account for the better recovery of regional function in some of the BMC patients. However, the fact that the number of grafts in the 'responder' and 'non-responder' group were similar, makes this interpretation unlikely. There was no significant difference in global LVEF between both groups at four months follow-up. The increase in LVEF postoperatively was comparable in both groups ( $41.2 \pm 10.1\%$  in controls vs  $45.8 \pm 13.2\%$  in the BMC group) and reflects the effect of revascularisation on the reversal of myocardial stunning. A further increase in LVEF, albeit not significant was observed at 4 months ( $43.1 \pm 10.9\%$  in control and  $48.9 \pm 9.5\%$  in the BMC group). This could be explained by the presence of hibernating myocardium.<sup>5</sup> Although  $^{201}\text{Thallium}$  uptake  $<50\%$  relative to the area of maximal tracer uptake, which defines nonviable scar tissue, has a high negative predictive value (90%), comparable to PET,<sup>6</sup> it can be expected that around 10% of nonviable segments would improve following revascularization alone. This could account for a further improvement in global LVEF after hospital discharge. In correspondence to our results, Janssens et al<sup>7</sup> reported no significant difference in global LVEF in a double-blind trial of intracoronary transplantation of autologous bone marrow. However, among patients treated within 6 hours of chest-pain onset, stem-cell therapy was associated with an almost 40% greater reduction in infarct size. No improvement in LVEF was observed in

patients with large infarcts treated with intracoronary mononuclear bone marrow cell transplantation.<sup>8</sup>

These findings are in contrast with a number of previous reports, in which improvement in global LVEF by transfer of bone-marrow derived cells was reported.<sup>2,3,9</sup> However, in those studies, BMC treatment was administered in the setting of acute myocardial infarction. Furthermore, in those studies baseline LVEF was systematically better preserved than in our patient population.

Our results showed significant improvement of systolic wall thickening between hospital discharge and 4 months follow-up in the BMC treated group. A possible confounding effect of CABG was ruled out since all affected segments in both groups were systematically revascularized. Moreover, Galinanes et al<sup>10</sup> have reported that transplantation of autologous bone marrow cells into scar tissue can only enhance cardiac function when used in combination with revascularisation. These results are not necessarily in contradiction with the observation that global LVEF did not significantly change. A critical factor of global LVEF recovery is the number of segments with improved wall motion. This number needs to represent approximately 20% of the left ventricle.<sup>11</sup> Given the relatively low dose of transplanted cells, it is unlikely that this number was reached in our study. Other trials, reporting significant improvement in global LVEF have used higher numbers of transplanted cells ( $245 \times 10^6$  in the TOPCARE-AMI trial<sup>2</sup>,  $24.6 \times 10^8$  in the BOOST trial<sup>3</sup>). This observation may warrant an investigation into a possible dose-response effect of BMC transplantation.

LVEDV did not decrease, indicating that BMC treatment did not improve left ventricular remodelling at 4 months follow-up.

Whether bone marrow derived stem cells can transdifferentiate to cardiomyocytes remains a matter of debate. Adult bone marrow contains a number of multipotential stem cells, such as mesenchymal and hematopoietic stem cells.<sup>12</sup> Orlic et al showed that in the mouse model



hematopoietic stem cells ( $\text{Lin}^- \text{c-kit}^+$ ) were able to differentiate into cardiac myocytes.<sup>1</sup> However, those results could not be reproduced: Murry et al transplanted  $\text{Lin}^- \text{c-kit}^+$  cells in MHC-nLAC mice.<sup>13</sup> These cells did not differentiate even 4 weeks after transplantation. Also Balsam et al reported that hematopoietic stem cells could not be found 30 days after injection into the ischemic myocardium of mice.<sup>14</sup> Nevertheless, Kajstura et al have recently repeated their previous observation that in the mouse model, in 10 days, nearly 4.5 million biochemically and morphologically differentiated myocytes together with coronary arterioles and capillary structures were generated independently of cell fusion.<sup>15</sup>

In view of the small size of this trial, subgroup analysis is difficult. However, the ‘responder’ group was transplanted with a cell population, containing a significantly higher percentage and absolute number of CD34+ cells than the non-responders. Hematopoietic stem cells and endothelial progenitor cells have been described as cells, expressing the hematopoietic marker CD34 on their surface. Those cells have the capacity to incorporate in sites of neovascularisation and differentiate into endothelial cells in situ.<sup>16</sup> Recently, using FDG as monitoring, Hofmann et al have shown that 14 to 39% of a CD34-enriched population homed into infarcted myocardium after intracoronary administration, whereas only 1.3 to 2.6% of an unselected BMC population did.<sup>4</sup> Those observations taken together suggest that CD34+ cells may play an important role in successful engraftment of BMC in infarcted cardiac tissue. Furthermore, no significant difference could be observed in other surface markers between the responder and non-responder group (Table 5).

The improvement in wall thickening in the BMC group was observed between hospital discharge and 4 months, suggesting that the time needed for differentiation of stem cells in myocardial infarction may be longer than expected. Indeed, Dai et al have shown that in the rat model mesenchymal stem cells did not express muscle-specific markers at 2 weeks but expression

started at three months in some animals and at 6 months in all animals.<sup>17</sup> Our group has transplanted mesenchymal (CD34-) cells into the infarct area at 4 hours after coronary occlusion in the sheep model. One month later, immunohistochemical staining for troponin-I and cardiac-specific myosin of transplanted mesenchymal stem cells was negative.<sup>18</sup>

Our study suggests that direct intramyocardial injection of bone marrow derived cells did not cause additional damage to the myocardium. The postoperative rise in markers for myocardial damage was not beyond that observed in the conventional CABG group. This may be in contrast with intracoronary administration: Vulliet et al have reported dose dependent ischemic changes and microinfarctions after intracoronary infusion of mesenchymal cells in noninfarcted dogs.<sup>19</sup> Kang et al reported a small increase in markers of myocardial damage after intracoronary infusion of peripheral blood stem-cells.<sup>20</sup> Other studies utilizing intracoronary administration of bone-marrow cells did not observe myocardial injury<sup>3</sup> nor microvascular dysfunction<sup>9</sup> after transient repetitive occlusion and simultaneous infusion of BMCs, or did not report specifically.<sup>2</sup> Occurrence of sustained ventricular tachycardia seems the only serious adverse event likely related to the procedure. Comparable to the study by Menasche et al<sup>21</sup> carried out with skeletal myoblasts, 6 patients had inducible VT. In the case of skeletal myoblasts, this problem was attributed to expression of different sets of ion channels. Furthermore, although functional gap junction formation between myoblasts/myotubes and neonatal rat cardiomyocytes has been reported in vitro, sustained coupling between engrafted myoblasts/myotubes and myocytes does not occur, creating electrically isolated islands.<sup>22</sup> Our findings contrast with at least one previous study of bone marrow cell transplantation in association with CABG, in which no serious ventricular arrhythmias were observed up to 14 months.<sup>23</sup> However, despite the fact that mesenchymal stem cells do express connexin-specific genes in co-culture with cardiomyocytes,<sup>24</sup>

occurrence of shortened refractory periods and increased slope of restitution were reported and can induce susceptibility to ventricular arrhythmia.<sup>25</sup>

Our study shows that autologous bone-marrow cell transplantation promotes regional left ventricular function in chronic heart failure patients. However, this did not translate into an increase in global left ventricular function, as reported in acute myocardial infarction patients. Future studies should be directed towards optimizing restoration of cardiac function.

### **CONFLICT OF INTERESTS**

The authors have no conflict of interests to disclose.

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**Table 1** : Patients' characteristics.

	<i>BMC group (n=10)</i>	<i>Control group (n=10)</i>	<i>p</i>
Age (years)	63.2 (8.5)	66.8 (9.2)	0.36
Male	10	7	0.06
Body-mass index	28.2 (3.1)	26.9 (3.6)	0.23
Diabetes	5	3	0.36
Cholesterol (mg/dl)	183 (35.8)	212 (37.8)	0.10
hypertension	7	5	0.36
current smoker	6	5	0.65
Infarct age (days)	217 (162)	213 (145)	0.95
Infarct area			0.80
anterior	2	1	
inferior	4	5	
inferobasal	2	1	
inferolateral	2	3	
# vessel (1/2/3) disease	0/2/8	1/2/7	0.76
Peak CK-total (U/L)	1308 (689)	824 (209)	0.09
Peak CK-MB (U/L)	48 (44)	40 (29)	0.73
cTnI (ng/ml)	6.6 (4.1)	9.7 (12.8)	0.68
number of grafts	2.8 (0.4)	2.5 (0.5)	0.07
Lima grafts	10	10	1
X-clamp time	48 (13)	47 (24)	0.52
perfusion time	109 (29)	87 (33)	0.25



**Table 2** : Characteristics of the BMC suspension.

Clinical data	BMC group	Control group
#cells harvested ( $\times 10^6$ )	60.25 (31.35)	41.44 (22.46)
% viability	95.05 (2.54)	97.99 (1.81)
% recovery	73.0 (14.6)	78.0 (14.6)
% CD34	1.42 (0.99)	2.00 (0.91)
CFU-GM/ $10^5$ MNC	76.37 (44.47)	113.64 (69.25)

**Table 3:** Global LVEF and left ventricular volume indices as determined by contrast-enhanced MRI.

	<u>Baseline</u>			<u>Postop</u>			<u>4M</u>			<u>Change(4M-Baseline)</u>		
	<i>Control</i>	<i>BMC group</i>	<i>p</i>	<i>Control</i>	<i>BMC group</i>	<i>p</i>	<i>Control</i>	<i>BMC group</i>	<i>p</i>	<i>Control</i>	<i>BMC group</i>	<i>p</i>
Global LVEF (%)	39.5 (5.5)	42.9 (10.3)	0.19	41.2 (10.1)	45.8 (13.2)	0.33	43.1(10.9)	48.9 (9.5)	0.25	3.6 (9.1)	6.1 (8.6)	0.41
LVEDVI (mL/m <sup>2</sup> )	89.4 (23.4)	86.9 (28.4)	0.76	87.6 (22.4)	87.0 (28.5)	0.97	92.8 (25.6)	87.1(20.4)	0.41	3.4 (28.2)	0.2 (35.0)	0.78
LVESVI (mL/m <sup>2</sup> )	54.1 (18.3)	49.6 (19.2)	0.24	51.5 (14.9)	47.1 (17.0)	0.5	52.8 (15.5)	44.5 (30.1)	0.57	-1.3 (14.1)	-5.1 (17.2)	0.27

**Table 4:** Total/ CD34+ cell number, Tl score and WT changes for individual transplanted patients.

	# cells transplanted (.10 <sup>6</sup> )	% CD34+ cells	# CD34+ cells transplanted (.10 <sup>6</sup> )	Tl defect score (4 M)	WT baseline (mm)	WT 4M (mm)
<b>RESPONDERS (R)</b>						
patient #1	21.18	6.06	1.284	1.0	-0.2 (2)	4.3
patient #2	41.00	1.19	0.486	2.5	-0.5 (4)	3.0
patient #3	127.26	2.99	3.805	3.5	-0.7 (6)	1.7
patient #4	48.03	3.74	1.796	2.6	0.2 (4)	4.4
patient #5	81.34	1.46	1.188	3.6	-1.5 (5)	2.8
mean (SD)	63.76 (41.59)	3.09 (1.97)	1.712 (1.260)	2.6 (1.0)	-0.6 (1.3)	3.0 (2.6)
<b>NON-RESPONDERS (NR)</b>						
patient #1	33.25	0.98	0.326	4.0	-1.9 (3)	0.8
patient #2	60.00	1.30	0.780	4.0	-0.4 (5)	-1.0
patient #3	38.53	0.75	0.289	4.0	0.0 (2)	0.9
patient #4	99.94	0.41	0.410	4.0	-0.8 (3)	0.3
mean (SD)	57.93 (30.30)	0.86 (0.38)	0.451 (0.225)	4.0 (0)	-0.7 (1.5)	0.0 (1.6)
p value (R vs NR)	0.82	0.03	0.05			
<b>DISCORDANT</b>						
patient #1	51.94	3.08	1.599	4.0	0.0 (1)	2.0

The numbers between brackets in the WT baseline column indicate the number of segments analyzed per patient.

**Table 5 :** Flowcytometric characterization of transplanted cells.

Marker	Non-responder	Responder
Hematopoietic cells		
CD38	68.4 (9.6)	64.0 (2.0)
CD117 (c-kit)	7.2 (3.5)	4.3 (2.6)
CD133 (AC-133)	3.7 (2.5)	4.6 (0.2)
HLA-dr	26.2 (10.3)	27.5 (5.5)
Endothelial cells		
CD105 (endoglin)	14.9 (6.1)	20.0 (2.0)
CD109	5.8 (3.3)	4.9 (0.2)
CD140b (PDGF-receptor b)	2.4 (1.5)	1.9 (0.9)
CD184 (CXCR4)	14.0 (5.4)	11.1 (4.9)
VEGFR-2 (KDR)	1.8 (1.3)	0.9 (0.1)
VEGFR-3	3.5 (4.2)	4.0 (2.6)
Mesenchymal cells		
CD29 ( $\beta$ 1-integrin)	21.9 (2.7)	23.0 (4.7)
CD44 (H-cam)	96.4 (2.0)	99.5 (0.5)
CD71	21.4 (7.2)	17.2 (5.9)
CD73 (SH4)	4.7 (2.1)	6.7 (2.7)

All data are expressed as % (SD).

## FIGURE LEGENDS

### Figure 1

Thallium-uptake defect score in bone-marrow cell (BMC) and control group. Only segments with preoperative score 4 (<50% uptake) with intention-to-treat were considered. The decrease in Tl-uptake was expressed as the sum score, adding up all segmental values. Preop = preoperative; postop = postoperative, at hospital discharge; 4M = 4 months follow-up. There was no significant difference between both groups ( $p = 0.51$  at discharge;  $p = 0.63$  at 4 M postoperative).

### Figure 2

Changes in wall thickening (systolic-diastolic wall thickness, in mm) as a function of time. PRE = preoperative; POST = postoperative, at hospital discharge; 4M = 4 months follow-up. At 4 months follow-up, a significant improvement was observed in the BMC group, whereas wall thickening remained unchanged in the control group ( $p = 0.007$ ). The interrupted lines in the BMC group indicate segments in patients, treated by amiodarone for inducible VT.

### Figure 3

Global LVEF change (4M-baseline) for each BMC patient, plotted as a function of the absolute number of CD34+ cells transplanted (in  $10^6$ ) ( $r=0.49$ ).

