# Intracoronary Stem-cell Injection after Myocardial Infarction: Microcirculation Sub-study

*Rodrigo de Carvalho Moreira, Andréa Ferreira Haddad, Suzana Alves Silva, André Luiz Silveira Souza, Fábio Antônio Abrantes Tuche, Mônica Amorim de Oliveira, Cláudio Tinoco Mesquita, Carlos Eduardo Rochitte, Radovan Borojevic, Hans Fernando Rocha Dohmann*

*Hospital Pro-Cardíaco[1], Botafogo; Secretaria Municipal de Saúde e Defesa Civil do Rio de Janeiro[2], Cidade Nova; Excellion - Serviços Biomédicos AS[3], Petrópolis, Rio de Janeiro, RJ, Brazil*

## Abstract

**Background: The injection of stem cells in the context of acute myocardial infarction (AMI) has been tested almost exclusively by anterograde intra-arterial coronary (IAC) delivery. The retrograde intravenous coronary (IVC) delivery may be an additional route.**

**Objetive: To compare the cell distribution and retention pattern in the anterograde and retrograde routes. To investigate the role of microvascular obstruction by magnetic resonance imaging in cell retention by cardiac tissue after the injection of bone marrow mononuclear cells (BMMC) in AMI.**

**Methods: This was a prospective, open label, randomized study. Patients with AMI who presented: (1) successful chemical or mechanical reperfusion within 24 hours of symptom onset and (2) infarction involving more than 10% of the left ventricle (LV) at the myocardial scintigraphy were included in the study. One hundred million BMMC were injected into the infarction-related artery through IAC route, or vein through the IVC route. One percent of the injected cells were labeled with 99mTc-hexamethyl-propylene-amine-oxime (99mTc-HMPAO). Cell distribution was evaluated at 4 and 24 hours after the myocardial scintigraphy injection. Cardiac magnetic resonance imaging was performed before cell injection.**

**Results: Thirty patients were randomized into three groups. There were no serious adverse events related to the procedure. The early and late retention of labeled cells was higher in the IAC group than in IVC group, regardless of the presence of microcirculation obstruction.**

**Conclusion: The injection using the retrograde approach was feasible and safe. Cell retention by cardiac tissue was higher using the anterograde approach. More studies are needed to confirm these findings. (Arq Bras Cardiol 2011;97(5):420-426)**

**Keywords: myocardial infarction/therapy; transplantation, autologous; bone marrow cells; stem cell transplantation/method.**

## Introduction

The microvascular damage caused by ischemia-reperfusion injury and stent implants results in spasm, embolization of debris, free radicals, inflammatory and necrotic cells, in addition to thrombus in the distal portions of the infarct-related artery[1]. In the early 1990s, it was demonstrated that a significant number of AMI patients had perfusion defects at tissue level, despite a normal TIMI flow after revascularization of the infarct-related artery. Since then, several studies have indicated that the presence of microvascular obstruction (MVO) is associated with increased mortality and worse prognosis after AMI[2-4].

**Mailing Address: Rodrigo de Carvalho Moreira •**
Praia do Flamengo, Flamengo – 22210030 - Rio de Janeiro – RJ - Brazil
E-mail: rodrigocm@cardiol.br
Manuscript received February 22, 2011; revised manuscript received May 05, 2011; accepted June 13, 2011.

It has been estimated that 30% of patients undergoing reperfusion have microvascular obstruction. This is particularly important because Janssens et al[5] found that half of patients undergoing autologous stem-cell transplant after AMI showed a microvascular obstruction pattern at the cardiac MRI, which may have impaired the migration of cells to cardiac tissue and hence, heart function improvement. Little is known about the retrograde percutaneous approach through the coronary vein, although this route has shown to be an effective way to deliver cells in animal human and models[6-8]. We suppose that the intravenous coronary (IVC) delivery can overcome the problem of MVO, because the diapedesis of circulating cells in the adjacent cardiac tissue occurs in the venous side of microcirculation[9].

The main objectives of this sub-study are: 1) to compare the pattern of cell distribution and retention in anterograde intra-arterial coronary (IAC) route, with the retrograde

intravenous coronary (IVC) route and 2) to investigate the role of microvascular obstruction detected by MRI in the retention of cells by cardiac tissue in autologous BMMC after AMI. We used radiolabelled cells to assess their distribution pattern in the heart, after they had been injected.

## Methods

Between January 3, 2005 and January 6, 2006, patients admitted at the Pro-Cardiaco Hospital and Hospital Municipal Miguel Couto (Rio de Janeiro) were selected for enrollment in the study if they were aged 18 to 80 years and had AMI. The patients selected at Hospital Miguel Couto were transferred to the Pro-Cardiaco Hospital, where all study-related procedures were performed. Patients were included if they: 1) had AMI with ST-segment elevation, defined as the presence of chest pain accompanied by ST elevation in two or more contiguous leads of a wall as well as enzymatic elevation, successfully reperfused with thrombolytic therapy or primary angioplasty within 24 hours of symptom onset, and 2) a fixed perfusion defect of more than 10% of LV mass after 72 hours at the single photon emission computed tomography (SPECT) with Technetium-99m methoxyisobutyl isonitrile (99mTc-MIBI) and sublingual nitrate.

Exclusion criteria were: 1) indication of coronary artery bypass surgery, 2) creatinine level> 2.0 mg / dl or hemodialysis, 3) infarct-related coronary artery with TIMI flow <3 by the time of cell injection; 4) sepsis, 5) persistent cardiogenic shock after 72 hours, 6) significant valve disease, defined as aortic stenosis with LV/Ao gradient > 50 mmHg, mitral stenosis with a valve area < 1.5 $cm^2$ or aortic and / or mitral regurgitation greater than moderate, 7) mechanical complications of AMI (myocardial rupture of the interventricular septum and LV free wall, papillary muscle rupture), 8) liver failure, and 9) severe pulmonary disease (COPD under continuous use of bronchodilators or steroids); 10) complete left bundle branch block or pacemaker rhythm (the SPECT imaging in the interventricular septum is compromised); 11) implanted pacemaker; 12) hematological diseases; 13) neoplasias, 14) other disorders of hemostasis and other diseases that could have an impact on life expectancy.

The Ethics Committee of Hospital Pro-Cardiaco (Rio de Janeiro) and the National Council on Ethics and Human Research (CONEP) (Brazil) approved the protocol. Informed consent was obtained from all patients.

### Study Design and Randomization

This was an open, randomized study of a series of consecutive patients. Between the third and sixth days after successful reperfusion of the infarct-related artery, enrolled patients were randomly divided into three groups: anterograde IAC approach, retrograde IVC approach and control. Randomization was stratified according to infarct size (≥ 25% or <25%), measured by scintigraphy at rest with sublingual nitrate, using "Quantitative SPECT Perfusion" imaging in three layers of different sizes (7, 5 and 3, respectively), with the use of sealed envelopes.

### BMMC Collection and Isolation

Approximately 80 ml of autologous bone marrow was aspirated from the posterior iliac crest of patients randomized to one of two treated groups. The procedure was performed under local anesthesia about 6 hours before the time scheduled for the injection procedure. The material was sent to the staff of the Federal University of Rio de Janeiro for handling. The cells were isolated by density gradient centrifugation in Ficoll-Paque Plus equipment (Amersham Biosciences, São Paulo, Brazil) and handled under aseptic conditions. They were also washed and resuspended in saline solution containing 5% human albumin, and finally filtered through a 100-micron nylon mesh to remove cell aggregates. A small fraction of the cell suspension was used for cell count and viability control using the trypan blue exclusion test. Cell viability was >90% in all subjects (93.26% ± 2.9).

### BMMC Delivery Techniques

The BMMC injection was performed 8.5 ± 1.44 hours after the bone marrow cell collection. The arterial access was performed via radial or femoral route. All patients received 10,000 IU of unfractionated heparin after sheath insertion. The intervention in vessels not related to infarction, but had indication for percutaneous treatment was performed before cell transfer in three patients in the IAC group and in one patient in the IVC group. Electrocardiogram, pulse oximetry, vital signs and clinical symptoms were monitored throughout the process.

Coronary angiography was performed in both IAC and IVC groups (GE Medical System, Advantx LCV plus, WI). A 6-F catheter guide was then placed in the ostium of the infarct-related coronary artery in order to confirm the patency of the target vessel and assess coronary blood flow (TIMI 3) before the cell injection.

In the IAC group, an over-the-wire (OTW) balloon catheter (Maverick ® Over-The-Wire balloon, Boston Scientific, Natick, MA), with a diameter 0.5 mm larger than the implanted stent was placed inside the stent previously placed in the culprit vessel, in order to temporarily stop the anterograde blood flow during infusions using the stop-flow technique[10]. Ten milliliters of solution containing 100 million autologous BMMC were infused through the central lumen of the balloon catheter during three consecutive coronary occlusions, lasting 2-3 minutes each, followed by 2 minutes of balloon deflation. One milliliter of solution containing labeled cells (see below) was diluted in 3 ml of saline solution and infused into the IAC as a final solution, using the same technique described before.

In the IVC group, access to the right internal jugular vein was used to position 5 or 6 Fr guide catheters, JR or multipurpose ones, in the coronary sinus, in addition to the arterial access. The same type of OTW balloon catheter (Maverick ® Over-The-Wire balloon), ranging from 3.5 to 4.0 × 9.0 mm in size was then advanced through the cardiac vein corresponding to the culprit vessel and positioned side by side with the balloon in the coronary artery, in the previously implanted stent. The total occlusion of the cardiac vein was then performed and maintained for at least 12 minutes. Four intermittent occlusions of the coronary arteries were performed simultaneously, in

order to reduce the washing of cells by the pressure of the anterograde flow. One milliliter of labeled cells (see below) was diluted in a solution containing 100 million mononuclear cells. During the first insufflation to stop the anterograde flow, 11 ml of the final cell solution were infused through the central lumen of the venous catheter balloon for one minute[11].

At the end of all procedures, a coronary angiography was performed as a control to verify the TIMI frame count pre-and post-PTA and cell transfer. Serial cardiac enzymes and ECG were performed before and after cell transfer.

### BMMC Labeling

In 12 patients from the IAC group and six from the IVC group, about 40 minutes before injection, a fraction of cells (1% of 108 cells) was incubated in sterile conditions in a 10 ml tube with 150 MBq of 99mTc-hexamethyl-propylene-amine-oxime (99mTc-HMPAO) (Amersham Biosciences, Piscataway, NJ) per $10^7$ cells for 30 minutes in a saline solution containing 2.5% human albumin.

The labeling efficiency was estimated after the removal of excess unbound radioactivity by washing the cells with saline with 2.5% human albumin. Radioactivity was measured with a dose calibrator (PTW Curiementor 2, Freiburg, Germany). Cell viability was assessed by exclusion with trypan blue.

Cells labeled with 99mTc-HMPAO were injected as described. The distribution in the tissue was observed with early and delayed whole-body imaging in anterior and posterior views, with 1024 x 256 pixels and 12 cm / min. For the topographic location of cells in the heart, chest CT images were acquired with a protocol similar to that used for myocardial perfusion imaging, 64 projections of 20 seconds each, with a resolution of 64 × 64 pixels. Images were reconstructed with a Butterworth filter, using the e-Soft 3.0 n software (Cedars Sinai QGS and Emory Cardiac Toolbox), and compared with the others acquired by perfusion CT.

All images were acquired using a dual-head gamma chamber (ECAM Duet, Siemens Medical Systems Inc, IL). Planar and SPECT images were obtained at 4 hours (3.67 ± 1.03) and 24 hours (22.8 ± 4.46) after the injection in all patients. Retention was defined as the percentage of the number of counts in anterior and posterior views of the heart (cardiac counts) in relation to the total number of counts of labeled cells in the body (body) in both early and delayed images. The decay rate was calculated by the following equation: [early (heart count/body count) - delayed (heart count/body count)] × 100/early (heart count/body count). The decay rate per hour was calculated by dividing the decay rate by the interval between retention of early and delayed images.

### Cardiac Magnetic Resonance

Cardiac magnetic resonance (CMR) imaging was performed 3-5 days after reperfusion. All studies were performed with commercially available software for MRI (GE Healthcare Milwaukee, WI, USA) as described before[12]. Briefly, the microvascular obstruction (MVO) was defined as delayed enhancement images taken early (within 2-5 minutes) after the injection of 0.20 mmol / kg gadopentetate dimeglumine (Gd-DTPA) through LV cross-sectional and longitudinal view in the same location used for the cine-MRI as a dark zone, subendocardial in the area of infarction (Figure 1). We defined infarct zone as a light signal in delayed enhancement images (10-20 minutes after contrast injection) in the inversion-recovery and gradient-echo sequences. All MRI studies were analyzed off-line in a workstation.

### Statistical Analysis

All imaging studies were analyzed by an independent experienced observer who was blinded to the allocation of patients to the two groups during the study. Continuous variables are shown as mean ± SD (unless otherwise stated). Categorical variables were compared by Fisher's exact test, whereas continuous variables were compared by Kruskal-Wallis test.

The rejection of the null hypothesis was based on a 5% significance, with a P value $<0.05$. All reported P values are two-tailed. The statistical analysis was performed using the SPSS software (version 13.0, SPSS Inc.).

## Results

Thirty patients were enrolled in the study: 14 in the IAC group, 10 in the IVC group and 6 in the control group. There was no significant difference in demographic parameters between the studied groups (Table 1). The period between AMI and cell injection was 5.5 ± 1.3 days and 6.1 ± 1.4 days in the IAC and IVC groups ($p = 0.14$), respectively.

The injections in IAC and IVC groups were performed successfully in all patients, except in one case in the IVC group, due to the tortuosity of the anterior interventricular vein. The distribution of cells labeled with 99mTc-HMPAO in the tissues was observed in the liver, lungs, spleen and other organs in addition to the heart (Figure 2). In the latter tissue, cell retention occurred in most cases, precisely in the infarcted area defined by the perfusion deficit. On scintigraphy images 4 hours after the BMMC injection, an average of 16.14% and 4.62% of cells were retained in the heart, when the IAC and IVC routes were compared, respectively ($p = 0.01$). After 24 hours, this percentage decreased to 10.29 in the IAC group and 3.13% in the IVC group (Chart 1). Microvascular Obstruction (MVO) was observed in 50% of the IAC group and in 60% of the IVC group. There was no difference in cell retention by the cardiac tissue regarding the presence of MVO in the groups (Chart 2).

## Discussion

The findings of the present study demonstrate that, although the anterograde and retrograde routes have shown to be viable and safe, the delivery of cells in the infarcted myocardium was more effective with the first technique, in terms of number of cells retained by the cardiac tissue, and

the fact that the microcirculation obstruction did not affect the number of cells retained in the infarcted myocardium in each group studied by myocardial scintigraphy.

In most studies of therapy with autologous stem cells for the treatment of AMI, the BMMC fraction was injected through the infarct-related artery after reperfusion, in order to reach the area of myocardium that was directly affected by blood flow interruption. This anterograde approach has proven to be feasible and safe. However, blood flow in the infarcted area can be severely affected, hindering the potential access of the injected cells into the area where they are needed for tissue repair. Therefore, the proposed alternative of retrograde intravenous approach is feasible and safe, but has been less used for the delivery of stem cells. A comparison between these two techniques was necessary to assess whether the possible presence of microvascular obstruction hindering the access of BMMC to the damaged tissue would make the retrograde approach the best option for cell delivery.

Our group has published a description of the first patient in this series[13]. An important characteristic of the studied routes was the large presence of labeled cells in other organs such as the lungs, probably due to the first-pass effect in organs related with hematopoiesis and lymphopoiesis, such as the liver and the spleen. Findings were also observed in pre-clinical studies of our and other groups[11,14]. The percentage of cells that were retained early in the IAC route (16.14%) is another noteworthy factor. Previous studies have reported that the rate of myocardial cell retention varied between 1% and 5% through IAC route[6,15,16].

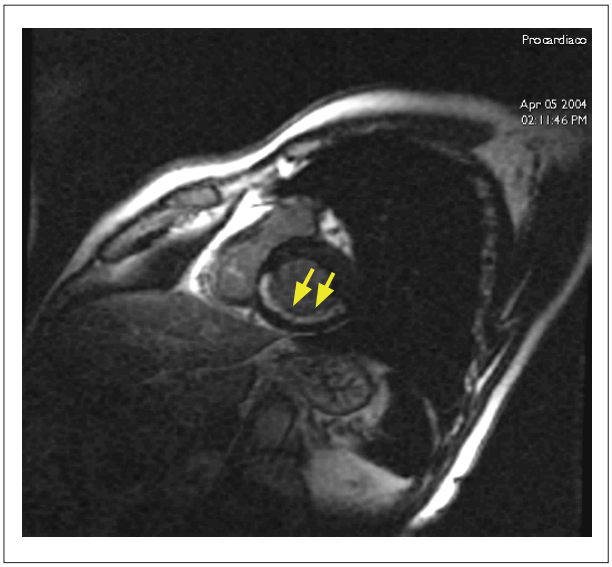


*Figure 1 - Cross-sectional view of the LV during cardiac magnetic resonance performed 3-5 days after the reperfusion, showing the presence of MVO (arrows).*

**Table 1 - Demographic data**

| Groups | Control | Intra-arterial | Intravenous |
|---|---|---|---|
| N | 6 | 14 | 10 |
| Age (years) | 57,2 ± 10,8 | 59,7 ± 14,3 | 53,6 ± 8,3 |
| Body Mass Index | 26,8 ± 4,9 | 25 ± 3,5 | 28,6 ± 4,0 |
| Male sex (%) | 4 (67%) | 10 (71%) | 7 (70%) |
| Diabetes (%) | 0 | 3 (21%) | 1 (10%) |
| Dyslipidemia (%) | 1 (17%) | 6 (43%) | 5 (50%) |
| Hypertension (%) | 2 (33%) | 11 (79%) | 6 (60%) |
| Smoking (%) | 1 (17%) | 8 (57%) | 2 (20%) |
| Family history of CAD (%) | 0 | 2 (14%) | 3 (30%) |
| Previous AMI (%) | 0 | 2 (14%) | 0 |
| Basal DR (%) | 29,5 ± 16,6 | 25,93 ± 14,1 | 35,4 ± 9,31 |
| **Coronary Artery Related to AMI** | | | |
| Anterior Descending (%) | 5 (83%) | 7 (50%) | 8 (80%) |
| Right Coronary (%) | 1 (17%) | 6 (43%) | 1 (10%) |
| Circumflex (%) | 0 | 1 (7%) | 1 (10%) |
| **PTA** | | | |
| Primary Angioplasty (AMI ≤ 12h) (%) | 1 (17%) | 4 (29%) | 2 (20%) |
| Late Angioplasty (AMI > 12h) (%) | 3 (50%) | 3 (21%) | 2 (20%) |
| Post-thrombolysis Angioplasty (AMI > 6h) (%) | 2 (33%) | 7 (50%) | 6 (60%) |

*CAD - coronary artery disease; DR - defect at rest; AMI - acute myocardial infarction; PTA - percutaneous transluminal angioplasty.*

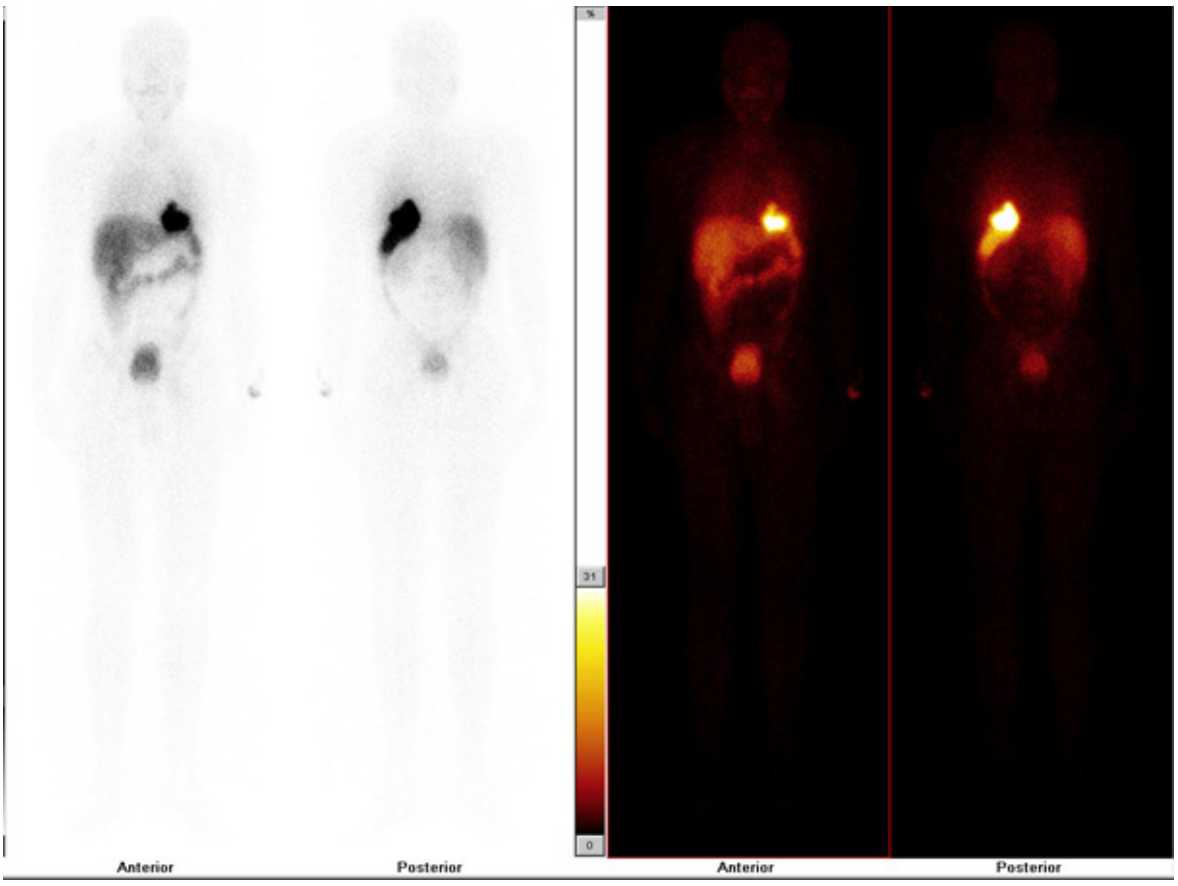


**Figure 2 -** *Total body scintigraphy in the anterior and posterior views, with BMMC labeled with 99mTc- HMPAO, showing the distribution of these cells in the heart, lungs and hematopoietic organs.*

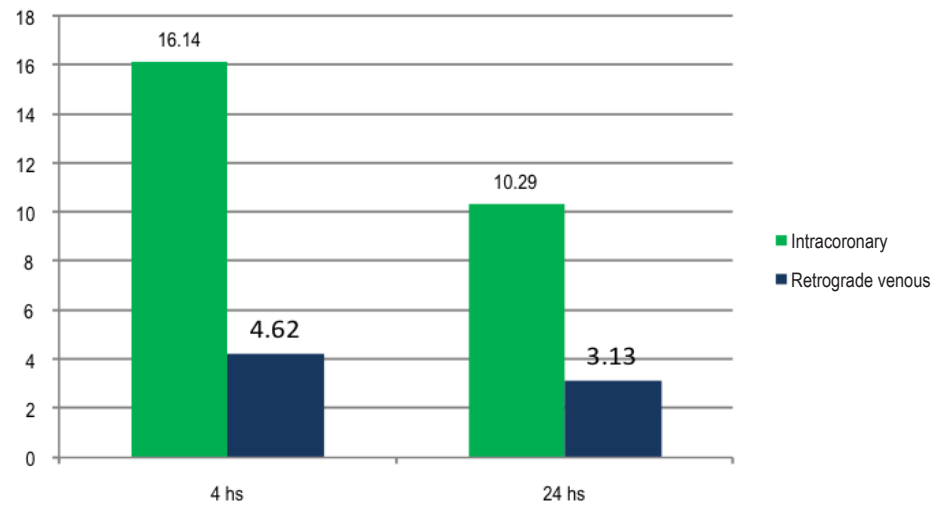


**Chart 1 -** *Uptake of BMMC labeled with 99mTc- HMPAO by the myocardium in relation to total body. Comparison of IAC and IVC routes.*

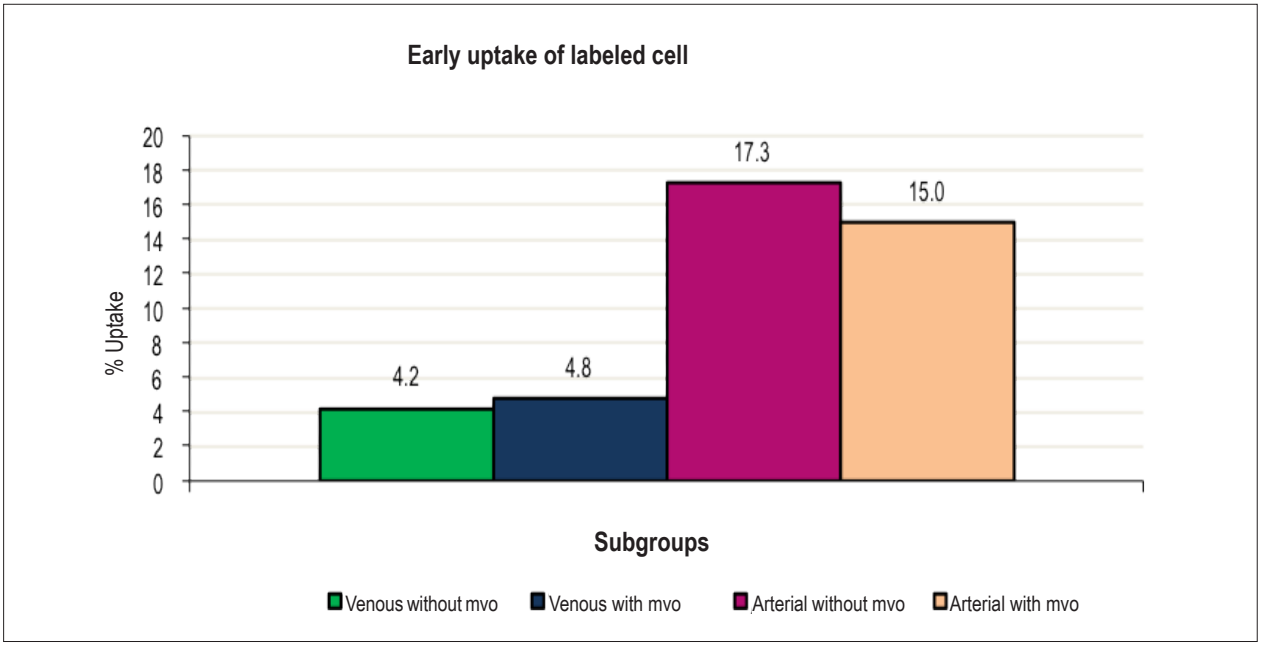


**Chart 2 -** *Early uptake (4h) of BMMC labeled with 99mTc- HMPAO by cardiac tissue. There was no difference regarding retention of labeled cells when comparing the IAC (p=ns) and IVC (p = ns) groups, according to the presence or absence of MVO. Retention was higher in the IAC group, regardless of the presence of MVO.*

Factors related to the type of tracer used and its half-life, time for cell injection and cell preparation may explain these findings. Janssens et al[5] performed injection procedures 24 hours after reperfusion, while we performed it, on average, five days later. Differences in the preparation of bone marrow cells can also influence cell quality and, therefore, retention and therapeutic benefit. We carried out the BMMC injections on the same day after preparation, 8 hours after collection. Other authors have used different protocols for isolation and storage, which may have implications in the migration and retention of these cells by the myocardium[17]. This study provides evidence that the BMMC adhesion and retention via IAC injection in the infarcted myocardium is feasible and safe, confirming previous findings on the mobilization signaling and homing of stem cells by the damaged tissue, during the period of acute ischemic lesion[18].

It also shows that the delivery via the IAC route was more effective in terms of cell retention by the myocardium than the IVC approach. This information is valuable, because a premise for therapy benefit is the graft of cells in the damaged tissue. New studies in cell therapies should improve the possible means, in order to release more cells in the region affected by ischemia. Possibly in the future, the choice of route will be individualized based on multiple criteria, for instance, cell type, type of heart disease, disease severity, comorbidities, inherent contraindications to the type of route and even choice based on patient preference.

This study was designed to answer questions on the release techniques and the consequent distribution of cells[19]. Its small sample size and its open-label nature prevent us from reaching definitive conclusions when evaluating subgroups[20]. We decided to label only a small fraction of cells (1%), in order to avoid unexpected negative effects on the cells, due to the higher total radiation. This small fraction may not reflect the overall cell distribution. Under the studied conditions, the anterograde route seems to have advantage over the retrograde one, but more studies are still needed, with the introduction of new parameters, such as, cell selection and association of growth factors[21,22].

## Conclusion

The injection using the retrograde approach showed to be feasible and safe. Cell retention by cardiac tissue was higher with the anterograde route. More studies are needed to confirm these findings.

## Acknowledgements

We thank Jader Cunha de Azevedo from Hospital Pro-Cardíaco for performing and evaluating the scintigraphy images, Vinicio Elia Soares from Hospital Municipal Miguel Couto for helping with patient recruitment and Ana Cristina Reis for being in charge of the administrative and regulatory aspects of the study.

### Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

### Sources of Funding

There were no external funding sources for this study.

### Study Association

This study is not associated with any post-graduation program.

## References

1. Sutsch G, Kiowski W, Bossard A, Luscher TF, Maier W, Vogt P, et al. Use of an emboli containment and retrieval system during percutaneous coronary angioplasty in native coronary arteries. Schweizerische medizinische Wochenschrift. 2000;130(33):1135-45.
2. Ito H, Maruyama A, Iwakura K, Takiuchi S, Masuyama T, Hori M, et al. Clinical implications of the 'no reflow' phenomenon: a predictor of complications and left ventricular remodeling in reperfused anterior wall myocardial infarction. Circulation. 1996;93(2):223-8.
3. Claeys MJ, Bosmans J, Veenstra L, Jorens P, De Raedt H, Vrints CJ. Determinants and prognostic implications of persistent ST-segment elevation after primary angioplasty for acute myocardial infarction: importance of microvascular reperfusion injury on clinical outcome. Circulation. 1999;99(15):1972-7.
4. Morishima I, Sone T, Okumura K, Tsuboi H, Kondo J, Mukawa H, et al. Angiographic no-reflow phenomenon as a predictor of adverse long-term outcome in patients treated with percutaneous transluminal coronary angioplasty for first acute myocardial infarction. J Am Coll Cardiol. 2000;36(4):1202-9.
5. Janssens S, Dubois C, Bogaert J, Theunissen K, Deroose C, Desmet W, et al. Autologous bone marrow-derived stem-cell transfer in patients with ST-segment elevation myocardial infarction: double-blind, randomised controlled trial. Lancet. 2006;367(9505):113-21.
6. Hou D, Youssef EA, Brinton TJ, Zhang P, Rogers P, Price ET, et al. Radiolabeled cell distribution after intramyocardial, intracoronary, and interstitial retrograde coronary venous delivery: implications for current clinical trials. Circulation. 2005;112(9 Suppl):I150-6.
7. Fearon WF, Ikeno F, Bailey LR, Hiatt BL, Herity NA, Carter AJ, et al. Evaluation of high-pressure retrograde coronary venous delivery of FGF-2 protein. Catheter Cardiovasc Interv. 2004;61(3):422-8.
8. Murad-Netto S, Moura R, Romeo LJM, Manoel Neto A, Duarte N, Barreto F, et al. Terapia de células-tronco no infarto agudo do miocárdio, através de perfusão coronariana retrógrada: uma nova técnica. Arq Bras Cardiol. 2004;83(4):349-51.
9. Liu L, Kubes P. Molecular mechanisms of leukocyte recruitment: organ-specific mechanisms of action. Thromb Haemost. 2003;89(2):213-20.
10. Strauer BE, Brehm M, Zeus T, Gattermann N, Hernandez A, Sorg RV, et al. Intracoronary, human autologous stem cell transplantation for myocardial regeneration following myocardial infarction. Dtsch Med Wochenschr. 2001;126(34-35):932-8.
11. Samoto VY, Branco ER, Ferreira G. Padrão de distribuição de células mononucleares da medula ósseaem tecido cardíaco sadio por diferentes vias de infusão. Rev Bras Cardiol Invas. 2009;17(2):220-6.
12. Rochitte CE, Lima JA, Bluemke DA, Reeder SB, McVeigh ER, Furuta T, et al. Magnitude and time course of microvascular obstruction and tissue injury after acute myocardial infarction. Circulation. 1998;98(10):1006-14.
13. Mesquita CT, Correa PL, Felix RC, Azevedo JC, Alves S, Oliveira CC, et al. Autologous bone marrow mononuclear cells labeled with Tc-99m hexamethylpropylene amine oxime scintigraphy after intracoronary stem cell therapy in acute myocardial infarction. J Nucl Cardiol. 2005;12(5):610-2.
14. Lu M, Zhao S, Liu Q, Jiang S, Song P, Qian H, et al. Transplantation with autologous mesenchymal stem cells after acute myocardial infarction evaluated by magnetic resonance imaging: an experimental study. J Thorac Imaging. 2011 Feb 17 [Epub ahead of print].
15. Freyman T, Polin G, Osman H, Crary J, Lu M, Cheng L, et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. Eur Heart J. 2006;27(9):1114-22.
16. Penicka M, Widimsky P, Kobylka P, Kozak T, Lang O. Images in cardiovascular medicine: early tissue distribution of bone marrow mononuclear cells after transcoronary transplantation in a patient with acute myocardial infarction. Circulation. 2005;112(4):e63-5.
17. Seeger FH, Tonn T, Krzossok N, Zeiher AM, Dimmeler S. Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction. Eur Heart J. 2007;28(6):766-72.
18. Spevack DM, Cavaleri S, Zolotarev A, Liebes L, Inghirami G, Tunick PA, et al. Increase in circulating bone marrow progenitor cells after myocardial infarction. Coron Artery Dis. 2006;17(4):345-9.
19. Silva SA, Sousa AL, Haddad AF, Azevedo JC, Soares VE, Peixoto CM, et al. Autologous bone-marrow mononuclear cell transplantation after acute myocardial infarction: comparison of two delivery techniques. Cell Transplant. 2009;18(3):343-52.
20. Nogueira FB, Silva SA, Haddad AF, Peixoto CM, Carvalho RM, Tuche FA, et al. Função sistólica de pacientes com infarto miocárdico submetidos a transplante autólogo da medula óssea. Arq Bras Cardiol. 2009;93(4):374-9.
21. Ghadge SK, Muhlstedt S, Ozcelik C, Bader M. SDF-1alpha as a therapeutic stem cell homing factor in myocardial infarction. Pharmacol Ther. 2011;129(1):97-108.
22. Mosna F, Annunziato F, Pizzolo G, Krampera M. Cell therapy for cardiac regeneration after myocardial infarct: which cell is the best? Cardiovasc Hematol Agents Med Chem. 2010;8(4):227-43.