

PROTOCOL FOR INTRACORONARY TRANSPLANTATION OF AUTOLOGOUS AC133 BONE MARROW STEM CELLS

**Stefan Iosif Dragulescu¹, Virgil Paunescu², Bogdan Mut-Vitcu¹,
Claudiu Plesa³, Adriana Plesa³, Erika Suci², Valentin Ordodi²,
Ioana Raluca Siska², Gabriela Tanasie², Carmen Bunu², Simona Popa⁴,
Andreea Dragulescu¹, Daniela Maximov¹, Ani Bokor¹, Ayman El-Kahlout¹**

REZUMAT

Obiective: Scopul studiului este de a evalua eficacitatea și riscurile transplantului autolog de celule stem, prin infuzie intracoronară, în artera corespunzătoare zonei de infarct la pacienții cu infarct miocardic acut sau recent. Studiile experimentale și clinice sugerează că transplantul de celule stem de origine medulară are un efect benefic asupra proceselor de remodelare a ventriculului stâng ce au loc după un infarct miocardic acut și contribuie la regenerarea miocardului afectat precum și la realizarea unei revascularizări de novo a miocardului ischemic.

Metode: În studiu au fost incluși 4 pacienți cu infarct miocardic masiv acut sau recent și cu realizarea reperuziei (PTCA cu implant de stent la nivelul coronarei afectate) și fracție de ejeție mai mică de 40%. Celulele medulare AC133+ au fost recoltate din creasta iliacă posterioară, apoi izolate, spălate și resuspendate pentru infuzie de cateter. Celulele au fost administrate intracoronar la nivelul arterei afectate de infarct. Investigațiile de rutină au inclus evaluare clinică și de laborator, EKG, monitorizare Holter pe 48 ore, ecocardiografie transtoracică, ecocardiografie de stres SPECT cu Technetiu 99 radioactiv, angiografie de ventricul stâng.

Rezultate: Nu s-au înregistrat complicații majore pre- sau postintervenție și nici aritmii maligne. Pacienții nu au mai prezentat angină sau semne clinice de agravare. La controlul efectuat la o lună de la intervenție nu au fost semnalate efecte adverse și nu s-au înregistrat aritmii maligne la monitorizare Holter pe 48 de ore.

Concluzii: Aceste rezultate sugerează că transplantul de celule stent autologe prin infuzie intracoronară este o metodă eficientă și sigură. Sunt necesare studii clinice pe termen lung pentru a atesta efectul benefic al acestei atitudini terapeutice.

Cuvinte cheie: celule stem, infarct miocardic, transplant autolog

ABSTRACT

Objectives: The aim of the study is to assess the safety and feasibility of autologous transplantation by intracoronary infusion of bone marrow stem cells into the infarct-related artery in patients with acute and recent myocardial infarction. Experimental and clinical studies suggest that transplantation of bone marrow derived stem cells beneficially affects left ventricular (LV) remodeling processes after acute myocardial infarction (AMI) and contributes to the regeneration of infarcted myocardium and neovascularization of ischemic myocardium.

Methods: We enrolled 4 patients with acute or recent (1 to 28 days), large myocardial infarction and successful reperfusion (percutaneous intervention (PCI) with stent implantation in AMI-related coronary artery) and LV ejection fraction < 40%. Bone marrow AC133+ cells were harvested from the posterior iliac crest, isolated, washed, and resuspended for injection by catheter. The cells were delivered by intracoronary injection into the infarct-related artery. Baseline investigations included clinical and laboratory evaluation, ECG, 48-hour Holter monitoring, transthoracic echocardiography, stress echocardiography, SPECT with Technetium 99 Sestamibi, LV angiography.

Results: No major pre- and postintervention complications occurred and no malignant arrhythmias on 48-hour Holter monitoring were noticed early after intracoronary infusion of bone marrow stem cells. All patients were free of angina and had no signs of clinical worsening. At one month follow-up no adverse cardiac events occurred and no malignant ventricular arrhythmias on 48-hour Holter monitoring were found.

Conclusions: These results suggest that autologous bone marrow cells transplantation by intracoronary infusion is a safe and feasible method. Long-term clinical studies are needed to attest the beneficial effects of this novel therapeutic approach.

Key Words: stem cells, myocardial infarction, autologous transplantation

INTRODUCTION

In patients with a large myocardial necrotic area resulting from acute myocardial infarction (MI), the loss of cardiomyocytes results in fibrous tissue synthesis and aneurysm formation, in left ventricular remodeling and subsequently, in progression of congestive heart failure.¹ The necessity of regeneration of myocardial contractile cells pool has encouraged the development of alternative methods to classical medical therapies.²

¹ Cardiology Clinic, Institute of Cardiovascular Medicine Timisoara

² Department of Physiology and Immunology

Victor Babes University of Medicine and Pharmacy Timisoara

³ 3rd Pediatric Clinic, Louis Turcanu Hospital, Timisoara

⁴ Euromedic Imaging Diagnostic Centre Arad

Correspondence to:

Stefan Iosif Dragulescu

Victor Babes University of Medicine and Pharmacy Timisoara

Piata Ioan Uta Colonel Martir no.2, 300041, Timisoara

Tel/Fax 256-216510; E-mail: rectorat@umft.ro

One of the most promising strategies is represented by autologous cell transplantation with either differentiated cells (skeletal myoblasts, cardiomyocytes) or stem cells.

Recent research suggests that stem cells from whole bone marrow possess greater functional plasticity than previously supposed.³ Since 1999, when Bittner et al.⁴ reported the possibility of cardiac muscle formation from circulating bone marrow cells, many studies have demonstrated the participation of stem cell to cardiac muscle formation in both animals and humans. In adult mice with experimental myocardial infarction, cardiomyocytes and vascular cells can be formed in vivo from circulating mouse bone marrow stem cells.^{5,6} Moreover, the stem cells also generate cardiomyocytes after direct injection into damaged heart tissue.⁷⁻⁹ In animal models pluripotential cells from bone marrow improved myocardial function and perfusion in the setting of ischemic heart disease.⁸ In addition, recent publications have described the beneficial effects (i.e., improvement in myocardial perfusion and segmental contractility) of autologous transplantation of mononuclear bone marrow cells immediately in the postinfarction period in humans.^{10,11}

All these data suggest that cardiomyocytes may be formed from bone marrow-derived hematopoietic^{6,8} and mesenchymal stem cells^{9,12}, but the extent of this process remains to be clarified by further studies.

Therefore, the aim of our study is to investigate the safety and feasibility of autologous transplantation by intracoronary infusion of bone marrow-derived stem cells into the infarct artery in patients with acute and recent myocardial infarction.

METHODS

CLINICAL DATA

Patients

Four patients were enrolled in this study after signing an informed consent approved by the Ethics Committee.

Inclusion criteria: age between 18 and 75 years, acute or recent myocardial infarction (1 to 28 days), left ventricular ejection fraction <40%, successful reperfusion – PCI with stent implantation in AMI related coronary artery.

Exclusion criteria: age over 75 years, cardiogenic shock (systolic blood pressure <80 mmHg requiring intravenous press or intra-aortic counterpulsation balloon), a history of leucopenia/thrombocytopenia, renal failure with serum creatinine > 2.5 mg%, evidence of malignant diseases, moderate/severe hepatic dysfunction, pregnancy, unwillingness to participate.

Baseline evaluation of the patients included a

complete clinical assessment (history and physical), laboratory evaluation (blood count, ASAT, ALAT), ECG, 48-hour Holter monitoring, transthoracic and stress echocardiography, SPECT with Technetium 99 Sestamibi, LV angiography. All patients received standard medical therapy at the time of enrollment.

The schedule of procedures in follow-up visits is summarized in Table 1.

Table 1. Follow-up time table of study patients

	Baseline	1 month	3 months	6 months	12 months
Clinical examination	+	+	+	+	+
Lab	+	+	+	+	+
ECG	+	+	+	+	+
Holter monitoring	+	+	+	+	+
ECO transthoracic	+	+	+	+	+
ECO stress	+	-	+	+	+
SPECT-Technetium 99 Sestamibi	+	-	+	+	+
LV-angiography	+	-	+	+	+

Early after the procedure, ECG and 48-hour Holter monitoring were performed. Serum CK, CK-MB, troponin T levels were assessed at 12 and 24 hours. Patients were monitored in the Cardiac Intensive Care Unit for 48 hours after the injection procedure.

Catheterization Procedure for Progenitor Cells Transplantation

The procedure consisted in advancing an over-the-wire balloon catheter into the stent previously implanted during the acute reperfusion procedure. To allow for adhesion and potential transmigration of the infused cells through the endothelium, the balloon was inflated with low pressure to completely block blood flow for 3 minutes, while 3 to 5 ml of the progenitor cell suspension was infused distally to the occluding balloon through the central port of the balloon catheter. This maneuver was repeated 2 or 3 times to accommodate infusion of a total of 10 ml cell suspension, interrupted by 3 to 5 minutes of reflow by deflating the balloon to minimize extensive ischemia. After completion of intracoronary cell transplantation, coronary angiography was repeated to establish vessel patency and the unrestricted flow of contrast media. The procedure was similar to that performed by other teams.^{11,12}

LABORATORY DATA

Bone Marrow Aspiration and Isolation of Stem Cells

A step-by-step diagram of the protocol for autologous stem cell transplantation, from harvesting to injection is presented in Figure 1.

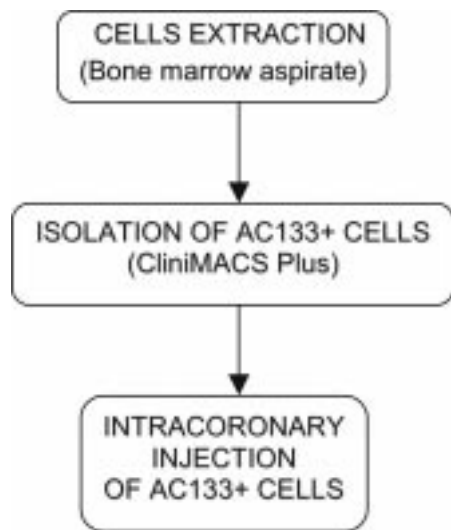


Figure 1. Steps to be followed for autologous stem cell transplantation.

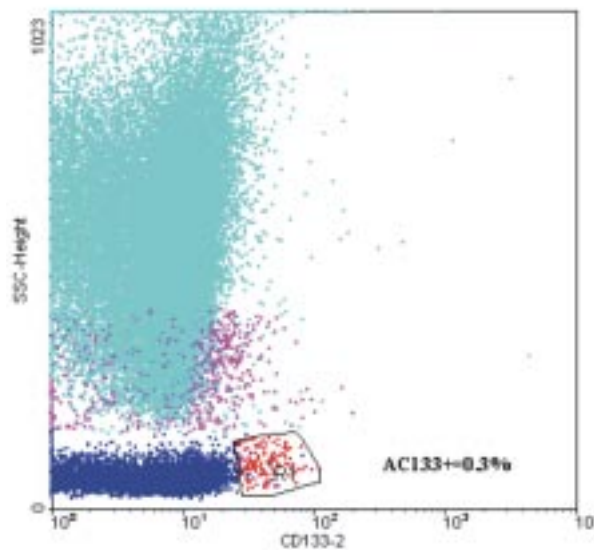
Bone marrow aspirates were obtained in the morning of the transplantation day. Approximately 12 hours before the cell injection procedure, bone marrow (600-650 ml) was aspirated from the posterior iliac crest under general anesthesia. Because of the high volume of bone marrow aspirate, all patients received an iso-group transfusion (500 ml).

The cells were isolated by centrifugation, and the buffy coat was used. Buffy coat cells were incubated with 5% human intravenous immunoglobulin and AC133 beads (MACS) for 30 minutes, at 20°C. Then, the cells were washed with Clini MACS buffer 0.5% human serum albumin, centrifuged, and finally resuspended in the same buffer (final volume = 90 mL). This volume was introduced in the Clini MACS Plus magnetic cell sorter (Milteny Biotech, Germany) for 90 minutes. The positive fraction oscillated from 4.5×10^6 to 8×10^6 AC133⁺ stem cells (Table 2). Cell viability was assessed using trypan blue exclusion.

Table 2. Characteristics of bone marrow cells injected

	Patient1	Patient2	Patient3	Patient4
BM aspirate (ml)	600	600	650	700
AC 133+ in BM aspirate (%)	0.16	0.2	0.3	0.14
AC 133+ Purity (%)	92	93	94	95
Recovery (%)	70.3	44	65	41
Viability (%)	98	97	97	97
AC133+ injected	4.5×10^6	6.5×10^6	8×10^6	5.5×10^6

The quantitative analysis of AC133⁺ stem cells was performed by immunophenotyping, using a flow cytometer FACS Calibur with 2 laser (Beckton-Dickinson), Cell Quest acquisition software, and Paint A Gate software for data analysis (Fig. 2, 3).



R1 = AC133+CD34+ cells (red dots)
Figure 2. Bone marrow aspirate before selection of AC133+ cells (patient 3)

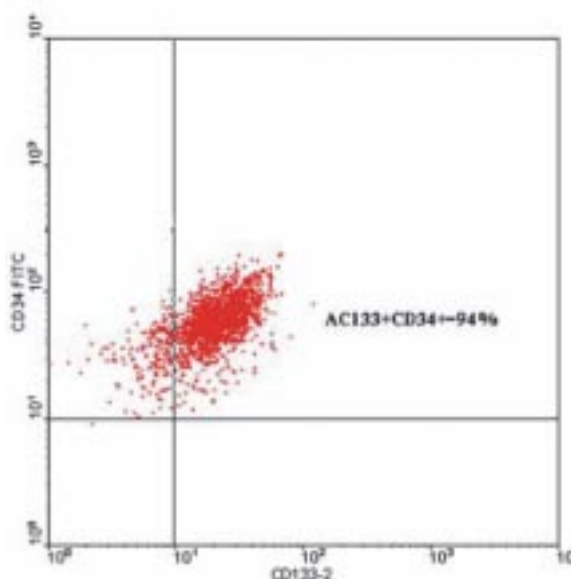


Figure 3. Positive fraction after selection of AC133+ cells using CliniMACS Plus sorter (patient 3).

RESULTS

Patient population baseline characteristics are presented in Table 3.

In all patients, baseline assessment of left ventricular ejection fraction (EF) and volumes was performed using SPECT. The values are showed in Table 4.

Post-transplantation results

No major pre- or post-intervention complications occurred (CK, CK-MB, T troponin levels were normal) and no malignant arrhythmias were found on 48-hour Holter monitoring. All patients were free of angina and had no signs of clinical worsening. They were discharged after 2-3 days of hospitalization.

Table 3. Baseline patient data

	Patient 1	Patient 2	Patient 3	Patient 4
Age	59	54	51	47
Gender	M	F	M	M
BMI (kg/m ²)	24.9	23.51	24.07	32
Hypertension	-	+	-	-
Diabetes	+	-	-	-
Hypercholesterolemia	+	+	+	+
Smoking	+	+	+	+
Family history of CHD	-	+	-	+
Previous MI	-	-	-	-
NYHA class	III	II	II	III
MI localization	Anterior	Anterior	Postero-inferior	Postero-inferior
Time from MI to cell therapy	28 days	20 days	13 days	4 days

Table 4. Baseline LV evaluation by SPECT with Technetium 99 Sestamibi

	Patient 1	Patient 2	Patient 3	Patient 4
EF(%)	27	39	35	38
EDV (ml)	187	176	127	124
ESV (ml)	137	106	82	76

Legend: EF-ejection fraction, EDV-end diastolic volume, ESV-end systolic volume.

1 Month Follow-Up Evaluation

After 1 month none of the patients had signs of clinical worsening. 48-hour Holter monitoring was negative for malignant ventricular arrhythmias.

DISCUSSIONS

The ability of stem cell transplantation to regenerate infarcted myocardium was shown to have considerable therapeutic potential by several researchers. Studies using autologous stem cell transplantation are summarized in Table 5.

Currently, it is unknown whether stem cell therapy would be more beneficial in early infarction or in its later remodeling phase, or even more valuable in the endstage of ischemic cardiomyopathy.¹⁷

Previous studies refer to clinical trials in both early

and late stages of an AMI. The cells used in these trials were different, ranging from unfractionated bone marrow cells to various populations of bone marrow stem cells; there are no data regarding the benefits of using a specific stem cell subpopulation (Table 5).¹⁸

We used AC133+ cells, which include a non-hematopoietic subpopulation of bone marrow stem cells that have a high potential to induce angiogenesis compared to unselected bone marrow cells.¹⁹ We chose this type of stem cells because we hypothesized that angiogenesis is the mechanism that allowed improvement in myocardial function in our patients. Moreover, some of the AC133+ cells belong to the MAPC (mature adult progenitor cells) population, which are capable of differentiation towards both visceral mesoderm cells, such as endothelial cells, and cells of limb-bud mesoderm, including myocytes. Therefore, using AC133+ cells might ensure both replacement of damaged myocardium and improvement of perfusion by stimulating neoangiogenesis.²⁰

First trials using intracoronary injection of mononuclear bone marrow cells injected in patients with acute and recent MI showed a significant improvement of parietal kinetics in the infarcted area and of contractility indices at 3-4 month follow-up.^{11,12}

Our data are similar to those described by Stamm et al.¹⁴ who delivered AC133+ stem cells in 6 patients who developed acute transmural myocardial infarction more than 10 days, but less than 3 months prior to admission, and were candidates for coronary artery bypass grafting (CABG). They reported good clinical results 3-9 months after surgery. All patients survived, with a significant improvement of perfusion in the previously non-perfused or hypoperfused infarct zone in 5 patients, and an enhanced global left-ventricular function in 4 patients. In our study, we aspirated larger amounts of bone marrow from the iliac crest (600-

Table 5. Summary of clinical trials on autologous transplantation of mononuclear bone marrow cells in humans with acute myocardial infarction

Research team	Patients	Cells	Route of administration	Outcome
Assmus et al., 2002 ¹¹	Post-MI (after 4.3±1.5 days)	Circulating or BM-derived progenitor cells	Intracoronary, into infarct related artery	Improved LV function and wall motion of infarcted area
Strauer et al., 2002 ¹²	Post-MI (after 5-9 days)	Mononuclear BM cells	Intracoronary, into infarct related artery	Improved myocardial perfusion and function, decreased infarct size
Hammano et al., 2001 ¹³	CABG candidates with post-MI scar	Mononuclear BM cells	Intramyocardial injection	Improved myocardial perfusion
Stamm et al., 2003 ¹⁴	CABG candidates with post-MI scar	AC133+ BM cells	Intramyocardial injection	Improved myocardial perfusion and LV function
Tse et al., 2003 ¹⁵	End-stage ischemic heart disease	BM cells	Intramyocardial injection	Improved myocardial perfusion and LV function
Perin et al., 2003 ¹⁶	End-stage ischemic heart disease	Mononuclear BM cells	Intramyocardial injection perfusion	Improved myocardial and LV function

Legend: BM = bone marrow, CABG = coronary artery bypass grafting, LV = left ventricular

700 ml, versus 85-195 ml in Stamm's trial) in order to isolate a higher number of AC133+ cells. We preferred to administer more AC133+ stem cells ($4.5-8 \times 10^6$ cells, compared to $1.21-3.37 \times 10^6$ cells in Stamm's trial), in order to make sure that a high number of cells will remain in the myocardium. Moreover, even though we used a similar cell sorter (CliniMACS), we obtained a higher purity, recovery and viability of the AC133+ cells than Stamm et al. (92-95%, 41-70.3%, and 97-98%, vs. 4-90%, 2-39%, and 75-91%).

In Stamm's trial, 3 of the 6 patients enrolled presented early complications (pneumonia, supraventricular tachycardia, pericardial effusion, bleeding); the patients included in our study were carefully selected and had no significant complications immediately after the procedure and at one month follow-up.

There are data which support the idea of a better way of delivery of stem cells in the heart, after myocardial infarction, by percutaneous catheter-based myocardial injections in several targeted ischemic regions. For example, Tse et al. recently demonstrated improvement in myocardial perfusion and segmental contractility after transendocardial injections of non-selected bone marrow cells.¹⁵ Similar results were reported by Perin et al.¹⁶ These preliminary data suggest that this procedure is relatively safe, underlining the fact that this alternative method improved both myocardial perfusion and function of the ischemic region, evidenced using MRI.

CONCLUSION

The preliminary results of our study suggest that intracoronary injection of AC133+ cells in patients with acute and recent myocardial infarction is safe and feasible. For the moment, the major limitations of this study are the small number of patients enrolled and the short term follow-up (one month), which limits conclusions about efficacy. Future analysis will be performed in this regard when longer-term follow-up data becomes available.

REFERENCES

1. Pfeffer MA. Left ventricular remodeling after acute myocardial infarction. *Annu Rev Med* 1995;46:455-66.
2. Siminiak T, Kurpisz M. Myocardial Replacement Therapy. *Circulation* 2003;108:1167-71.
3. Forbes SJ, Vig P, Poulsom R, et al. Adult stem cell plasticity: new pathways of tissue regeneration become visible. *Clinical Science* 2002;103:355-69.
4. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med* 2001;7:430-36.
5. Orlic D, Hill JM, Arai AE. Stem cells for myocardial regeneration. *Circ Res* 2002;91:1092-102.
6. Bittner RE, Schofer C, Weipoltshammer K, et al. Recruitment of bone-marrow-derived cells by skeletal and cardiac muscle in adult dystrophic mdx mice. *Anat Embryol (Berlin)* 1999;199:391-6.
7. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997;275:964-7.
8. Schattman GC, Hanlon HD, Jiao C, et al. Blood-derived angioblasts accelerate blood-flow restoration in diabetic mice. *J Clin Invest* 2000;106:571-8.
9. Takahashi T, Kalka C, Masuda H, et al. Ischemia- and cytokine-induced mobilization of bone marrow derived endothelial progenitor cells for neovascularization. *Nat Med* 1999;5:434-8.
10. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest* 2001;107:1395-402.
11. Assmus B, Schächinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). *Circulation* 2002;106:3009-17.
12. Strauer BE, Brehm M, Zeus T et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 2002;106:1913-21.
13. Hamano K, Nishida M, Hirata K, et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ J* 2001;65:845-7.
14. Stamm C, Westphal B, Kleine HD, et al. Autologous bone marrow stem cell transplantation for myocardial regeneration. *Lancet* 2003;361:5-46.
15. Tse HF, Kwong YL, Chan JKF, et al. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet* 2003;361:47-9.
16. Perin EC, Dohmann HFR, Borojevic R, et al. Transendocardial autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation* 2003;107:2294-302.
17. Hagege AA, Vilquin J-T, Bruneval P, Menasché P. Regeneration of the myocardium. A new role in the treatment of ischemic heart disease? *Hypertension* 2001;38:1413-19.
18. Caplice NM, Gersh BJ. Stem cells to repair the heart: a clinical perspective. *Circ Res* 2003;92:6-8.
19. Reyes M, Dudek A, Jahagirdar B, et al. Origin of endothelial progenitors in human postnatal bone marrow. *J Clin Invest* 2002;109:337-46.
20. Laham RJ, Oettgen P. Bone marrow transplantation for the heart: fact or fiction? *Lancet* 2003;361:11-2.