**Improvement of Cardiac Function by Intracoronary Freshly Isolated Bone Marrow Cells Transplantation in Patients With Acute Myocardial Infarction**

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**Background:** We analyzed in the present study the influence of intracoronary autologous freshly isolated bone marrow cells transplantation (BMCs-Tx) on cardiac function in patients with acute myocardial infarction (AMI).

**Methods and Results:** The 32 patients with AMI were enrolled in this prospective nonrandomized study to either freshly isolated BMC-Tx or to a control group without cell therapy. Global left ventricular ejection fraction (LVEF) and the size of infarct area were determined by left ventriculography. We observed in patients with autologous freshly isolated BMCs-Tx at 6 months follow up a significant reduction of infarct size as compared to control group. Moreover, we found a significant increase of LVEF as well as infarct wall movement velocity at 6 months follow up in cell therapy group as compared to control group. In the control group there was no significant difference of LVEF, infarct size and infarct wall movement velocity between baseline and 6 months after AMI.

**Conclusions:** These results demonstrate for the first time that intracoronary transplantation of autologous freshly isolated BMCs by use of a point of care system is safe, and may lead to improvement of cardiac function in patients with AMI. (Circ J 2011; 75: 683–691)

**Key Words:** Acute myocardial infarction; Freshly isolated bone marrow cell transplantation; Global left ventricular ejection fraction; Infarct size

Despite widespread use of primary percutaneous coronary intervention for prompt reperfusion of the infarcted myocardium, acute myocardial infarction (AMI) is a major cause of chronic heart failure (HF). HF is a leading public health problem resulting in increased risk of cardiovascular complications and mortality. Despite advances in medical therapy, the prevalence of HF is still increasing. Conventional medical strategies as well as an increase of chronic rejection after heart transplantation for the treatment of HF due to myocardial infarction (MI) do not attempt to correct the underlying cause (ie, loss of viable or functional myocardial tissue), raising a need for strategies aimed at myocardial regeneration and repair. Autologous bone marrow cell transplantation (BMCs-Tx) is a promising novel option for treatment of cardiovascular disease. In animal models, bone marrow-derived stem/progenitor cell infusion improves cardiac function and neovascularization after MI. Additionally, recent clinical studies provide further evidence for a promising improvement of cardiac function after intracoronary infusion of BMCs in patients with AMI. However, it is unknown whether freshly isolated BMCs transplantation have beneficial effects postinfarction remodeling. In this prospective nonrandomized control trial, we analyzed the influence of intracoronary freshly isolated cell therapy by use of point of care system on cardiac function in patients with AMI.

**Patients’ Characteristics**

In a prospective nonrandomized controlled trial, 32 patients between 18 and 80 years of age were eligible for inclusion in this study if they had had an acute ST-elevation MI on the...
The point of the study was the change in left ventricular ejection fraction (LVEF) as well as the size of infarcted area measured by left ventriculography after 6 months. The secondary endpoint was the functional status by NYHA classification in both groups. All data were obtained by blinded expert readers unaware of patient group assignment.

**Coronary Angiography and Left Ventriculography**

All patients in both groups underwent left heart catheterization, left ventriculography and coronary angiography. Cardiac function and infarct size were determined by left ventriculography. Cardiac function was evaluated by LVEF and by auxotonic myocardial contractility index, evaluated by the wall movement velocity of the infarcted area. LVEF was measured with Quantcor software (Siemens, Erlangen/Germany). To quantify the size of infarct area we used the centerline method according to Sheehan et al. by plotting 5 axes perpendicular to the long axis of the heart in the main akinetic or dyskinetic segment of ventricular wall. Systolic and diastolic lengths were then measured by 2 independent observers, and the mean difference was divided by systolic duration in seconds. The follow-up was 6 months after the treatment. All hemodynamic investigations were obtained by 2 independent observers. All data were obtained by blinded expert readers unaware of patient group assignment.

**Preparation and Administrations of BMC**

Seven days after AMI, a total of 120 ml bone marrow was taken from the iliac crest after local anesthesia and mononuclear cells were isolated and identified including CD34+ and CD133+. The cell suspension concentration consisted of a heterogeneous cell population including hematopoietic, mesenchymal and other progenitor cells and processed according to manufacturers instructions (using Bone Marrow Aspirate...
Concentrate BMAC™, Harvest Technologies GmbH, Munich, Germany).

After undergoing arterial puncture, all patients received 7,500–10,000 U units of heparin. Cell transplantation was performed via intracoronary administration of 4–6 fractional infusions of 3–5 ml cell suspension. All cells were infused directly into the infarcted zone through the infarct-related artery via an angioplasty balloon catheter, which was inflated at low pressure (2–4 atm) and was located within the previously stented coronary segment. This prevented back flow of cells and produced stop flow beyond the site of balloon inflation to facilitate high-pressure infiltration of cells into the infarcted zone with prolonged contact time for cellular migration. Six months after catheter-guided cell transplantation, all functional tests were repeated, including coronary angiography and left ventriculography. There were no procedural or cell-induced complications and there were no side effects in any patients.

Safety Parameters
To assess any inflammatory response and myocardial reaction after cell therapy, white blood cell count, and the serum levels of both C-reactive protein (CRP) and creatine kinase (CK) were determined immediately before as well as after treatment. Additional analysis was done directly after transplantation and 3 months later: BNP level in PB, ECG at rest, 24-h Holter ECG and echocardiography.

Statistical Analysis
Quantitative data are presented with mean±SD and qualitative data are tabulated using absolute frequencies and/or percentages. Differences between therapy groups for qualitative variables are tested using Fisher’s-Exact-Test due to the small number of patients in each therapy group. Within differences of quantitative variables in each therapy group were compared using the Wilcoxon-Test for depending samples, and differences between therapy groups of quantitative variables were compared with the Wilcoxon-Test (Mann-Whitney-Test) for independent samples. Both of the nonparametric Wilcoxon-Tests are preferred due to the more likely expected non-normal distribution of the data. For all statistical tests, the result was seen as statistically significant if the corresponding 2-sided \( p \)-value was smaller or equal to 0.05. Statistical analysis was performed with SPSS for Windows (Version 15.0). Analysis of the intra- and interobserver variabilities of endpoint measurements was performed using correlation analysis and the Spearman correlation coefficient (Statistical software package SAS Version 9.2) as well as the method of Bland-Altman assessment of agreement. More than 95% of inter- or intra-rater difference was within the “limits of agreement” defined as mean±2SD, if plotted against the mean differences of a patient’s individual data.

Results
Baseline Characteristics of the Patients
We enrolled 32 patients with AMI after acute coronary revascularization in the study. Of these, 17 patients in the first group received autologous freshly isolated BMCs-Tx into the infarct-related coronary artery, and 15 patients in the

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<th>Table 2. Cardiac Function, Clinical Function Status Parameters at Baseline and 6 Months After AMI in the BMCs-Tx Group</th>
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<td>The size of infarct area (%)</td>
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<td>LVEDV (ml)</td>
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<td>SVI (ml/min)</td>
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Values are mean±SD. There were no significant differences in baseline cardiac function, clinical function status parameters between 2 groups. LVEDV, left ventricular end-diastolic volume; LVE ESV, left ventricular end-systolic volume; SVI, stroke volume index; NYHA, New York Heart Association. Other abbreviations see in Table 1.

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<th>Table 3. Cardiac Function, Clinical Function Status Parameters at Baseline and 6 Months After AMI in Control Group Without BMCs-Tx</th>
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Values are mean±SD. There were no significant differences in baseline cardiac function, clinical function status parameters between 2 groups. Abbreviations see in Tables 1, 2.
second group received no intracoronary BMCs transplantation. There were no significant differences between the baseline characteristics and demographics of patients in both groups (Table 1).

Cellular Composition of Point of Care System From BMCs

Table 4 shows the cellular composition of the bone marrow aspirate (120ml) and bone marrow concentrate (20ml) as well as the viability of cells by use of point of care system. The number of total nucleated cells, CD34+, CD133+, and platelet counts increased significantly post separation in the bone marrow concentrate compared to pre-separation in the bone marrow aspirate (P<0.001).

Effect of Freshly Isolated BMCs Transplantation

Left Ventricular (LV) Function, Infarct Size and Infarct Wall Movement Velocity

LVEF, LV end-diastolic and end-systolic volumes (LVEDV, LVESV, respectively), stroke volume index (SVI), infarct size and the wall movement velocity of the infarcted area were measured by left ventriculography in the first group at baseline and 6 months after BMCs-Tx as well as in the second group without BMCs-Tx.
Freshly Isolated BM Cells Transplantation in AMI

There were no significant baseline differences in LVEF, infarct size or infarct wall movement velocity between the 2 groups. There was a significant decrease of infarct size 6 months after cell transplantation compared to control group without cell therapy. Moreover, no significant changes were observed in the control group at follow-up. Additionally, Panel B shows the decrease of single values of infarct size after BMCs-Tx for each patient in cell therapy group. AMI, acute myocardial infarction; BMCs-Tx, bone marrow cells transplantation.
Functional Status and Clinical Safety Parameters

To determine the functional status we assessed NYHA classification in both groups by 2 independent and blinded physicians. There were no significant baseline differences in NYHA classification between the 2 groups. Intact wall movement velocity significantly increased 6 months after cell therapy as compared to control group. Furthermore, no significant changes were observed in the control group at follow-up. Additionally, Panel B shows the increase of single values of intact wall movement velocity after BMCs-Tx for each patient in cell therapy group. AMI, acute myocardial infarction; BMCs-Tx, bone marrow cells transplantation.

Figure 3. (A, B) Infarct wall movement velocity were measured by left ventriculography immediately before and 6 months after procedure in both groups. There were no significant baseline differences in intact wall movement velocity between the 2 groups. Intact wall movement velocity significantly increased 6 months after cell therapy as compared to control group. Furthermore, no significant changes were observed in the control group at follow-up. Additionally, Panel B shows the increase of single values of intact wall movement velocity after BMCs-Tx for each patient in cell therapy group. AMI, acute myocardial infarction; BMCs-Tx, bone marrow cells transplantation.
Discussion

In this prospective nonrandomized controlled study we examined the influence of autologous freshly isolated intracoronary BMCs-Tx on the LV function in patients with AMI after 6 months.

Despite widespread use of primary percutaneous coronary intervention for prompt reperfusion of the infarcted myocardium, AMI is a major cause of chronic HF. The risk of chronic HF as well as mortality and morbidity are significantly increased in patients with reduced LVEF after AMI. The use of stem cell-based therapy is becoming increasingly recognized as having the potential to salvage damaged myocardium and to promote endogenous repair of cardiac tissue, thus having the potential for the treatment of HF. In animal models autologous infusion or injection of stem/progenitor cells derived from various sources was shown to enhance blood flow and neovascularization and improve heart function after MI. Moreover, clinical pilot and randomized studies suggested, that the intracoronary infusion of autologous BMCs is safe and feasible in patients with AMI. While initial pilot studies by Strauer et al and Fernandez-Aviles et al as well as the TOPCARE-AMI and BOOST trials reported an improvement in LVEF and improved perfusion in the infarcted area 4–6 months after cell transplantation, a randomized controlled trial by Janssens et al did not reveal a significant effect on LVEF, but showed an improvement in regional EF and a reduction of the infarct size in the BMC group. The beneficial effects observed in most phase II/III studies were confirmed in the so far largest double-blind, randomized multicenter REPAIR-AMI trial. Only one larger study, the ASTAMI trial, did not show any benefit on left ventricular functional parameters. The reason for the failure of the ASTAMI trial to show a benefit of cell therapy may have been the different cell isolation and storage protocol, which significantly affected the functional capacity of the cells. Whereas in the REPAIR-AMI trial Ficoll gradient centrifugation was used for cell isolation, the negative clinical ASTAMI trial used a different, not yet validated, technique (LymphoPrep). Strikingly, the yield in total nucleated cells out of the same volume of 50 ml bone marrow aspirate was quite different. While the Ficoll-based protocol, which was used for the isolation procedure in the REPAIR-AMI trial, provided 3-fold higher number of cells as compared to ASTAMI trial. Even more importantly, recent data also suggest that the number of hematopoietic colony-forming units and the SDF-1-induced migratory activity of recovered BMCs based on the ASTAMI protocol are significantly lower compared to the Ficoll protocol. These data suggest that, although similar techniques were used, the functional activity and/or cellular composition of the obtained cellular product were quite different. Because most of the previous clinical trials involved BMCs isolated by Ficoll, this technique is currently viewed as the gold standard. Our findings that the infarct size reduced, whereas the LVEF and regional infarct wall movement velocity increased, 6 months after intracoronary cell therapy in patients with AMI, are in line with the data of previous published pilot and randomized clinical trials. Additionally, we observed improvement of the functional status (NYHA classification) 6 months after cell therapy. Cell isolation procedures are crucial for the functional activity of the administered cellular product. In our trial we chose to use a point of care system for the preparation of the treating cell composition. We demonstrated the same results for the first time with intracoronary freshly isolated BMCs-Tx using a point of care system with Harvest BMAC-system for the
preparation of the treating cell composition, unlike many previously conducted trials that employed Ficoll gradient separation as the method of cell collection, which produces a very limited cell lineage spectrum. Harvest BMAC system is a point of care system for the concentration of BMCs. The cellular composition of the concentrate, which was prepared by the Harvest BMAC system, differs from that prepared using the Ficoll method. The Ficoll composition contains predominantly mononuclear cells (lymphocytes, erythroblasts and monocytes) and very few granulocytes. The Harvest BMAC system concentrates the entire nucleated cell population with mononuclear cells and specific stem cell populations (CD34+ and CD133+) from the marrow aspirate as well as the platelets (Table 4). Importantly, however, the Harvest device provided an advantage of producing a significantly higher yield of isolated BMCs compared to the Ficoll protocol. Thus, if the number of infused cells in the in vivo neovascularization model was adjusted for this higher yield of BMCs, the treatment effect was significantly greater compared to Ficoll BMCs, as assessed by limb perfusion measurement.29 One obvious difference in the 2 compositions is the presence of significant numbers of granulocytes and platelets in the Harvest BMAC composition. Platelets and granulocytes have been shown to have a positive effect on the neovascular potential of the resulting concentrate. The presence of platelets within the composition could be important because it has been shown that these platelet-derived mediators also potently enhance postnatal angiogenesis. Iba et al demonstrated that implantation of mononuclear cells together with platelets into ischemic limbs more effectively augments collateral vessel formation by supplying various angiogenic factors, in which VEGF played a key role.29 Indeed, Massberg et al provided compelling evidence that platelets generate the critical signal that recruits CD34+ BMCs and c-Kit+Sca-1+Lin- bone marrow-derived progenitor cells to sites of injury.29 Therefore, these findings strongly support the notion that implanted platelets play a pivotal role in stem and progenitor cell recruitment, and provide a rationale for the fact that Harvest BMAC produced functional in vivo results similar to or better than Ficoll. In our study, despite higher number of platelets, we observed no immediate periprocedural or postprocedural adverse complications. In addition, unlike Ficoll isolation where cells are resuspended in a serum free medium, Harvest BMAC is always resuspended in the patient’s own plasma. Thus, the isolated cells are not removed from their natural plasma microenvironment, which may be helpful to sustain the functionality of the cells. This has been further supported by experimental study of Hermann et al,24 who showed that the BMAC composition to be significantly more bioactive than the Ficoll composition. Intriguingly, however, due to the greater yield of cells generated by the Harvest device, the cellular product isolated from a given bone marrow aspirate by the Harvest BMAC device may actually translate into even greater therapeutic effects. Additionally, practical aspects may also deserve consideration. Importantly, a major limitation of the Ficoll isolation procedure for clinical applications is that it is strongly investigator dependent, immensely time consuming and requires a good manufacturing practice (GMP) facility. In this study, we were able to demonstrate that such complex methods are not necessary to achieve established results. As the concentration process by use of a point of care system is completed with a 15-min period, everything can be accomplished in one session without adding excessive time to the overall procedure, circumventing the previously mentioned disadvantages of the Ficoll isolation process. The Harvest device not only provides a much shorter turnaround time, but it also does not require an expensive GMP facility for the cell isolation procedure. Therefore, this device represents a cost-effective and time-efficient stand-alone technique for the isolation of autologous BMCs suitable for cell therapy regimens in the rapidly growing field of regenerative medicine.

Several hypotheses have been proposed about how intracoronary cell therapy improves myocardial function. Experimental studies addressing the capacity of transplanted bone marrow-derived stem cells to differentiate into the cardio-myogenic lineage yielded conflicting results.32–34 Recent well-conducted studies suggest that the BMCs do not transdifferentiate into cardiomyocytes but adopt mature hematopoietic characteristics. In contrast to embryonic stem cells, most adult stem or progenitor cells do not spontaneously differentiate into cardiomyocytes, but rather require an adequate stimulus to do so. Another proposed mechanism is that cell therapy may increase angiogenesis and improve blood supply to ischemic regions, potentially aiding in the revascularization of hibernating myocardium and preventing cardiomyocyte apoptosis. Additionally or alternatively, the local microenvironment plays an important role to induce cell fate changes by physical cell-to-cell interaction or by providing paracrine factors promoting tissue repair.32–34 Cell-based therapy is a promising option for treatment of ischemic disease. However, cell therapy is in its early stages, and various questions remain. For example, the mechanisms of action by which cells exhibit beneficial effects. Currently, a variety of autologous adult progenitor cells are undergoing preclinical evaluation. BMCs are, at present, the most frequent source used clinically for cardiac repair.35 BMC fractions include a heterogeneous mixture of cells with varying percentages of hematopoietic stem cells, BM-CPCs, mesenchymal stem cells, and side population cells.36,37 In the present study, we could demonstrate that intracoronary transplantation of autologous freshly isolated BMCs by use of a point of care system improved LVEF and reduced infarct size significantly in patients with AMI after 6 months.
Moreover, we observed a significant enhancement of NYHA classification, even 6 months after cell transplantation. This interesting observation could be implemented in future large-scale randomized studies.

References


