

# TRANSPLANTATION OF BONE MARROW DERIVED PROGENITOR CELLS IN ACUTE MYOCARDIAL INFARCTION. THE FIRST RESULTS

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**Summary:** The intracoronary administration of autologous bone marrow cells (BMCs) has been shown to improve the left ventricle function in the course of acute myocardial infarction.

Therefore we have started a clinical trial using transplantation of BMCs in the acute phase of myocardial infarction. The aim of our study is to assess the feasibility and safety of this procedure, and effect on the left ventricle function of these patients. We describe the first experience in two patients with acute myocardial infarction reperfused using direct stenting. The aspiration of bone marrow from the sternum provided sufficient amount of the cells for transplantation. No serious ischemia and no changes in coronary artery patency were detected after intracoronary infusion. The left ventricle ejection fraction was increasing throughout the time of three-month follow-up. No other complications (ventricular arrhythmias, reinfarction, thrombus formation) were detected.

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**Key words:** Myocardial infarction; Bone marrow cells; Progenitor cells; Ejection fraction

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## Introduction

The first results of the clinical studies using the intracoronary administration of bone marrow progenitor cells (BMC) after successful revascularization in the course of acute myocardial infarction (MI) are promising (2). It has been reported that improvement of the left ventricle function is caused by the stimulation of neovascularization and the enhancement of cardiac myocyte regeneration in the infarcted area (1,2,3,4,5,6). The previous observations and clinical studies have shown that this method is feasible and safe (9).

The aim of our study is to investigate the feasibility, safety, and initial clinical outcome of intracoronary infusion of autologous progenitor cells in patients with acute MI. The present report describes our first experience with this method.

## Material and Methods

### Patients

Two patients over 18 years with ST-segment elevation extensive acute MI treated with direct stenting are included

into the study. Exclusion criteria are the presence of cardiogenic shock (defined as the presence of hypotension systolic blood pressure < 80mmHg requiring catecholamine treatment or intraaortic balloon counterpulsation), women in reproductive age, haematologic diseases, major bleeding requiring blood substitution, serious liver and kidney diseases, any malignant disease in the past or at present, immunodeficiency, viral or bacterial infection, or unwillingness to participate. The study protocol was approved by The Ethics Committee of the University Hospital in Hradec Králové, and the study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from both patients.

### *Preparation and characterization of transplanted cells, transplantation protocol*

Fifty mL of bone marrow aspirate was obtained from the sternum into a sterile plastic container with 7 ml of ACD-A solution (Baxter SA, Belgium). Nucleated bone-marrow cells were isolated by density gradient sedimentation in 6% dextran with ACD-A solution (ratio 6:1) followed by centrifugation of isolated cells in the same solution. The final cell concentration was adjusted by diluting with dex-

tran ACD solution (mentioned above) and divided into 3 syringes each containing 3 mL of suspension of bone marrow nucleated cells. Cell manipulation was made in a clean room with validated grade A with the background B. The cell suspension consisted of heterogeneous cell populations including hematopoietic progenitor cells and other cell types (eg. side population cells, stroma cells, etc.). Hematopoietic progenitor cells were determined by flow-cytometry analyses using directly monoclonal antibodies against anti-human CD 34 (QBend PE, Immunotech Marseille, France) and CD 133 (QBend PE, Miltenyi Biotec GmbH, Germany) as well as by culture of CFU-GM and BFU-E. Mesenchymal cells were cultured as well. After ex-vivo preparation, the aspirate was transplanted within 24 hours. Cells were infused via an over-the-wire balloon catheter advanced into the stent previously implanted during the acute reperfusion procedure. The amount of 3mL of progenitor cell suspension was infused during low-pressure balloon inflation with the aim to completely block blood flow for 2-3 minutes and to allow for adhesion and transmigration of the infused cells through the endothelium. The mononucleated cell doses were  $3,72 \times 10^7$  and  $2,35 \times 10^7$ , number of CFU-GM was  $2,34 \times 10^5$  and  $1,29 \times 10^5$ , respectively.

This maneuver was repeated three times to accommodate infusion of the total 12 and 15mL respectively progenitor cell suspension, interrupted by 3 minutes of reflow by deflation of the balloon to minimize the extension of ischemia. After completion of intracoronary cell transplantation, coronary angiography was repeated to ascertain vessel patency and unimpeded flow of contrast material.

Before and after procedure, plasma levels of creatine-kinase, MB, and troponin T were assessed with the aim to analyse the presence of serious myocardial ischemia.

The function and regional motion defects of the left ventricle were assessed using echocardiography which was performed before, 1 week, one, and three months after the procedure.

### Preliminary results

The study started in October 2004 when the first two patients were treated using the progenitor cell transplantation. Both patients (males, aged 37 and 54 years, first manifestation of coronary artery disease, no positive family history) were admitted to hospital for ST-segment elevation antero-septal MI. Direct PCI (percutaneous coronary intervention) with stent implantation into left anterior descending coronary artery was done within 6 hours after the onset of the chest pain. In both cases, the full restoration of the blood flow was achieved (TIMI flow III). Echocardiography showed the extensive antero-apicolateral area of akinesis with EF < 40%. Both patients were treated using standard medication (aspirin, clopidogrel, statin,  $\beta$ -blocker, and ACE inhibitor) during their hospitalization.

The transplantation of the progenitor cells was performed between days five and ten of AMI course (day 7 and

9 of the course of MI). The procedure was well tolerated with only mild angina at the time of the balloon inflation and the infusion of the progenitor cells. In both patients, the material for progenitor cell suspension was aspirated from sternum (three punctures, 35mL of aspirate, four punctures, 45mL of aspirate respectively). There were no procedural complications (no ventricular arrhythmia, thrombus formation, distal embolization, coronary artery occlusion or dissection). During hospitalization, no ventricular arrhythmia was documented. Troponin T and CK as a marker of myocardial necrosis were assessed before and after 24 h, and one week after the cell transplantation. Both markers were decreasing during the time of observation. There were no significant changes in blood count.

Both patients completed three-month follow-up without any complications. Holter monitoring was performed during the ambulatory follow-up and did not reveal any significant arrhythmia.

The function of the left ventricle was assessed using echocardiography. Echocardiographic parameters showed improvement. Ejection fraction was increasing in both patients (from 35 to 40%, resp. from 40 to 50%).

### Discussion

The capacity of adult bone marrow cells to convert into other tissues, referred as plasticity, is the background of their potential use in many fields of clinical medicine (8). Recent experimental studies revealed that bone marrow derived progenitor cells might contribute to the regeneration of ischemic myocardium and can enhance neovascularization (5).

The major goal of the intracoronary delivery of the autologous bone marrow cells is to improve the function of the left ventricle not only by the stimulation of neovascularization, but also by the enhancement of cardiac myocyte regeneration in the infarcted area (6,10).

The data of TOPCARE-AMI Trial (2,9) showed that intracoronary infusion of progenitor cells (either bone-marrow derived or circulating progenitor cells) is safe and feasible in patients after acute MI which has been treated using direct coronary artery stenting. This study also showed favourable effects on the left ventricle remodelling.

At this time, there is no possibility to postulate detailed conclusions for the cell therapy in acute MI. But our observation showed that this therapy was feasible and safe in both patients with acute MI. We did not observe any complications associated with the procedure. In both patients this treatment led to the increase of ejection fraction. Our observation also showed that the sternum can be used for aspiration of sufficient count of bone marrow cells.

In a view of the fact, that there are still many questions, which have to be answered, there is need of further investigation at this area.

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