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Stem Cell Therapy in Myocardial Infarction Clinical Point of View and the Results of the REANIMA Study (REgenerAtion of Myocardium with boNe Marrow Mononuclear Cells in MyocArdial Infarction)

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1. Introduction

The incidence of heart failure (HF) after acute myocardial infarction (AMI) is around 10-40% during the hospital stay depending on its definition (Weir & McMurray, 2006; Cleland & Torabi, 2005). Also, another 10-20% of patients will develop heart failure symptoms during the next few months and years (Torabi et al., 2008). The mortality of patients with heart failure symptoms after AMI is very high and it reaches up to 50% in 5 years (Weir & McMurray, 2006; Fox et al, 2006). The left ventricle dilatation occurs in even 30% in patients reperfused successfully with primary angioplasty during six months follow-up (Bolognese el al, 2002) and the occurrence of dilatation is more pronounced in patients with lower baseline left ventricle ejection fraction (LVEF). The incidence of HF after AMI has increased, and mortality decreased over time with the better reperfusion therapy (Velagaleti et al, 2008). According to these facts, it is extremely important to develop therapeutic modalities in order to prevent the remodeling of myocardium after infarction. The adult stem cell therapy is a relatively new and promising method of an infarcted heart healing and HF prevention.

In the last two decades three important discoveries regarding different regenerative steps of damaged myocardium promoted the completely new era in the treatment of ischemic heart disease. First of all, several adult multipotent and pluripotent stem cells from different tissues may trans-differentiate in certain circumstances to cardiomyocytes or other needed cells, such as endothelial cells (Körbling M & Estrov Z, 2003; Müller et al, 2005). However, in vivo, this mechanism of heart regeneration seems to be negligible (Wagers et al, 2002; Murry et al, 2004), at least for the acute injury. The second is the fact that a significant number of cardiac cells are in the proliferative state in the areas of myocardium adjunction to infarction (Beltrami et al, 2001). The first source of these regenerative cells is very probably resident cardiac stem cells which are in the quiescent state out of injury, but in the time of infarction they proliferate and differentiate to cardiomyocytes, smooth muscle cells and endothelial cells (Bollini et al, 2011). And the third important discovery is that in the time of infarction,

myocardial ischemia initiates the eruption of cytokines, growth-factors and chemokines from the injured myocardium which promote mobilization of stem cells from other niches and their homing into the damaged myocardium (Frangogiannis, 2008). The most likely function of these cells in the ischemic myocardium are various paracrine effects which enable survival of severely damaged cardiomyocytes, promote differentiation and the proliferation of cardiac stem cells and participate in the creation of new blood vessels which all halted myocardial remodeling and the development of heart failure (Mirotsou et al, 2011).

The knowledge of these processes is very important because the regenerative therapy depends on artificial augmentation of some steps in order to make regenerative process more efficient. The most important steps are shown in figure one. Ischemic injury induces the hypoxia-inducible factor-alpha which in turns stimulates the expression of several growth factors and chemokines in the infracted heart (Dong et al, 2010). Those cytokines, especially stromal derived factor-1, interleukin-8 and vascular-endothelial growth factor promote mobilization of local and remote stem cells and enable engraftment of them into the damaged tissue (Figure 1).

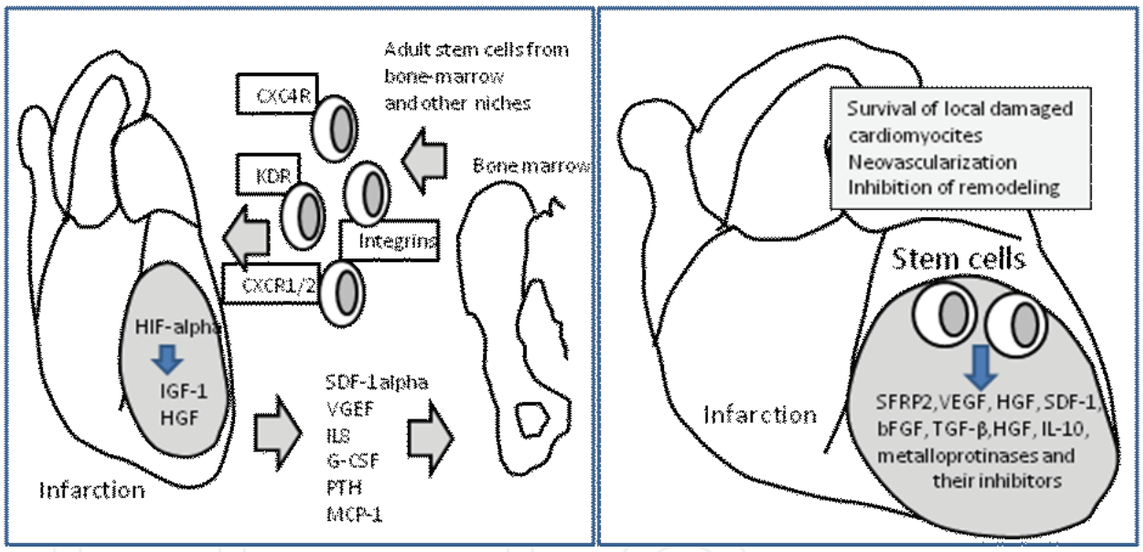

Fig. 1. Mobilization of stem cells by the cytokine and chemokine storm after myocardial infarction and potential paracrine effect of stem cells in the infracted heart and beneficial effect on cardiomyocytes survival, promotion of angiogenesis and inhibition of remodeling HIF-α – hypoxia inducible factor-alpha, IGF-1 – insulin-like growth factor – 1, HGF – hepatocyte growth factor, SDF-1alpha – stromal cell-derived factor 1 alpha, VEGF - vascular endothelial growth factor, G-CSF- granulocyte colony-stimulating factor, IL8 - interleukin 8, PTH - parathyroid hormone, MCP-1 – monocyte chemoattractant protein-1, KDR – receptor for VGEF, CXC4R – receptor for SDF-1, CXCR1/2 receptors for other chemokines, SFRP2 – signaling protein important for cardiomyocyte survival.

Chemokine receptors (CXC-R1 and CXC4R), growth receptors (VGFR) and several selectins and integrins on stem cells are important for the successful homing of these cells in the ischemic myocardium (Chavakis et al, 2008). Expression of matrix metalloproteinases such as MMP-2, 9 and cathepsin by stem cells represent the final step of their transmigration into

the damaged tissue (Cheng et al, 2007; Huang et al, 2009). Several growth factors up-regulated by ischemia (insulin growth factor-1, hepatocyte growth factor, fibroblast growth factor) enable the survival of these cells in the hostile environment (Frangogiannis, 2008). Paracrine effects of stem cells promote local cardiomyocytes survival, neovascularization, attenuate the remodeling and improve cardiac function. Among several niches of stem cell residency, myocardium itself, bone marrow and adipose tissue are probably the most important reservoir of this regenerative capacity. The advance age, large necrosis and enhanced inflammatory reaction decrease the stem cell mobilization after infarction (Turan et al, 2007).

2. Important clinical trials on stem cell therapy in acute myocardial infarction

Several clinical studies investigated the usage of bone marrow derived cells for the treatment of AMI. The most of them used autologous bone marrow derived mononuclear (MNC) cell suspensions with intracoronary delivery through the inflated balloon placed on the spot of previous stent placement (Abdel-Latif et al, 2007; Tongers et al, 2011). The pioneering study of Strauer (Strauer et al, 2002), on 20 AMI was not randomized, but had the well matched control group that showed improved left ventricular systolic function and perfusion in the short and long-term follow-up. After that study several randomized studies were published with the conflicting results (Table 1). Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE study) compared bone marrow derived MNC and circulating progenitor cells (CPS) given intracoronary but without the control group (Schachinger et al, 2004). Both systolic function and viability improved in the similar way after 4 months follow-up. In the study of Chen et al (Chen et al, 2004) intracoronary injections of mesenchymal stem cells were used for the first time in humans, and with the sophisticated methodology they demonstrated that this method was safe, feasible and that it significantly improved global and regional left ventricle function. Interestingly, there was no trial with the use of MSC intracoronary after Chen's study. In BOO transfer to enhance ST-elevation infarct regeneration (BOOST) trial (Schafer et al, 2006) with magnetic resonance imaging (MRI) of left ventricle ejection fraction (LVEF) and volumes for follow-up, single dose of intracoronary bone marrow cell provided the accelerate improvement of systolic function (after 6 months) with the late catch-up of the control group (after 18 months). In the study of Janssens et al, intracoronary transfer of bone marrow MNC was done 24 hours after primary percutaneous coronary intervention (PCI) and did improve only regional, but not the global left ventricle systolic function after 4 months by the MRI imaging (Jansenss et al, 2006). Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) trial (Schachinger et al, 2006a, 2006b) is the largest randomized trial that examined the intracoronary transfer of bone marrow derived MNC and it brought interesting results. For the first time one of the inclusion criteria for the participation in the study was the baseline LVEF measured at the time of primary PCI. The significant improvement of LVEF was detected in the cell therapy group compared to controls and it was more pronounced in patients with the baseline LVEF less than median (48.9%) and in those in whom cell transfer was performed later than the 4-post infarction day. The most important result of this trial was that the combined end point death and recurrence of myocardial infarction and rehospitalization for heart failure, was significantly reduced in the BMC group after two years follow-up (Assmus et al, 2010).

Method of SC delivery	Number of patients and type of cells N	Timing (d)	Bone marrow volume, method of cell preparation and the number of cells	Criteria for patient selection	The basic result 4-6 months after STEMI
I.C. short FU					
Strauer et al.	10 BMMNC/10 C	5-9	40 ml, Ficoll, 2.8±2.2x10^7 MNC	First STEMI, pPCI	ECHO, LVA, PET - EF, volumes, perfusion ↑
TOPCARE	29 BM-MNC/30 CPC	4-6	50 ml, Ficoll 5±3x10^6 CD34+/16±12x10^6CPC	First STEMI, pPCI	ECHO, RVA, MRI – EF, volumes, perfusion↑
Chen	34 BM-MSC/35 C	8/16 Harv/ deli	60 ml, MSC culture 8-10x10^9 MSC	First STEMI, pPCI	ECHO, PET – EF, volumes, perfusion↑
BOOST	30 BMC/30 C	5-7	120 ml, gelatin-polysuccinate, 9.5±6.3x10^6 CD34+	First STEMI, pPCI	MRI - EF↑ at six but not at 18 months
Jansens	33 BMMNC/34 C	1	130 ml, Ficoll, 2.8±1.7x10^6 CD34+	First STEMI, pPCI	ECHO, MRI - EF↔, regional function↑
REPEAR-AMI	101 BMMNC/103 C	3-6	50 ml, Ficoll, 3.6±3.6x10^6 CD34+	First STEMI, pPCI, EF≤45%	LVA - EF↑ Comp hard end point↓
ASTAMI	50 BMMNC/47 C	4-7	50 ml, Lymphoprep, 0.7x10^6 CD34+	First STEMI, pPCI on LAD	ECHO-EF, SPECT, MRI – EF and volumes↔
Meluzin	22 HD-BMMNC/22 LD-BMMNC/22 C	5-9	NS, Histopaque-buffy-coat, HD-10^8 MNC, LD-10^7 MNC	First STEMI pPCI	ECHO, gSPECT - ↑EF, ↓volumes, HD better
REGENT	80 NS-BMMNC/80 CD34+/CXC4R+BM Cells/40 C*	3-12	100-120 ml-selected cell group and 50-70 ml-unselected group, Ficoll/selection 1.8x10^8 cells/1.9x10^6 CD34+CXCR4+	First STEMI, LAD-IRA, EF≤40%	MRI – EF and volumes↔, EF and volumes↑ in pts with EF<37% (median)
FINCELL	40 BMMNC/40 C	2-6 (after PES stent)	80 ml, Ficoll, 2.6±1.6x10^6 CD34+	First STEMI, Fibrinolysis	ECHO, LVA - EF↑ IVUS - MLA↔
HEBE	69 BMMNC/66 PBMNC/ 65 C	3-8	60 ml BM, 150-200 ml PB, Lymphoprep, 4.0(2.1-6.5)x10^6 CD34+/0.3(0.2-0.4)x10^6 CD34+	First STEMI, pPCI	MRI - EF, IS and regional function↔
I.C.long FU					
BALANCE	62 BMMNC/62 C	5-10	80-120 ml, Ficoll, 6.1±3.9x10^7 BMC	First STEMI, pPCI	LVA, dECHO - EF↑, arrhythmias↓, mortality↓

Method of SC delivery	Number of patients and type of cells N	Timing (d)	Bone marrow volume, method of cell preparation and the number of cells	Criteria for patient selection	The basic result 4-6 months after STEMI
CAO	41 BMMNC/46 C	7	40 ml, Lymphoprep,5x10^8MNC (1.8±0.6%CD34+)	First STEMI, pPCI on LAD	gSPECT - EF↑, viability↔
BOOST 5y	27 BMMNC/26 C	5-7	120 ml, gelatin-polysuccinae, 9.5±6.3x10^6 CD34+	First STEMI, pPCI	MRI – EF and volumes↔
Repeated I.C.					
Yao	12 S-i.c.BMMNC transfer/15 R-i.c.BMMNC - 3 months/12 C	7 d and 90 d	90 ml, Ficoll, 1.9-2.1x10^8 BMC in both groups and in repeat infusion	First STEMI, EF 20-39%	MRI EF↑ highest in repeat cell group
I.V.					
Hare	39 alloMSC (0.5 vs 1.6 vs 5.0x10^6/kg) /21 C	1-10	Single unrelated donor no HLA matched	First STEMI, pPCI	ECHO-EF antMI↑, MRI-EF↑
Endocardial					
MYSTAR	30 EG/30 LG	3-6 w vs. 3-4 m	300 ml, COBE-vol. depl. EG: 3.6x10^6 CD34+i.m. +23.2.4x10^6CD34+i.c. LG: 3.0.3x10^6 CD34+i.m. +22.5x10^6CD34+ i.c.	First STEMI, pPCI, 30-45%EF	SPECT-EF↑ in both groups, no difference between groups

SC- stem cells, I.C.- intracoronary, I.V. intravenously, FU- follow-up, BMC-Bone marrow cells, BM-MNC – Bone marrow mononuclear cells, CPC-circulating progenitor cells, Bone marrow mesenchimal stem cells, HD-MMNC – higher dose of BMMNC-10^8, LD-BMMNC-lower dose BMMNC-10^7, PBMNC-peripheral blood mononuclear cells, C-controls, PES- paclitaxel eluting stent, STEMI- ST elevation myocardial infarction, pPCI- primary percutaneous coronary intervention, antMI – anterior myocardial infarction, LAD- left anterior descending, IA- infarction related artery, EG – early group, LG – late group, dECHO- dobutamine echocardiography, gSPECT- gated single-photon emission computed tomography, PET- positron emission tomography, MRI- magnetic resonance imaging, LVA- left ventricle angiography, EMM- electro-mechanical-mapping, IVUS- intravascular ultrasound, MLA-minimal lumen area, EF- ejection fraction.

Table 1. Important clinical trials of stem cell therapy in acute myocardial infarction.

Autologous Stem-Cell Transplantation in Acute Myocardial infarction trial (ASTAMI) also used some inclusion criteria for attention to recruit more severe seek patients (Lunde et al, 2006). The inclusion criterion in this study, among the presence of the first STEMI, was the finding on coronarography with the culprit lesion on the proximal part of the left anterior descending artery (LAD). However, more than 25% of patients in both groups (cell group and control) had the TIMI-2/3 flow before the primary percutaneous coronary intervention (PCI) and the baseline mean LVEF measured by three methods (echocardiography, single photon computed tomography-SPECT and MRI) was greater than 40%, which means that this group did not represent the anterior STEMI realistically. This study showed no effects of cell therapy on global LVEF. The other probably important pitfall of this study was the late baseline MRI imaging, after 3 weeks of stem cell infusion which could have missed some

early action of stem cells. Different protocols of bone marrow mononuclear cell preparation (for instance - Lymphoprep gradient media in ASTAMI and Ficoll in REPAIR-AMI) among the studies might be the reason for these discrepant results, but there are certain controversies about that issue (Seeger et al, 2007; Yeo et al, 2009). Meluzin et al, addressed the question of "cell dosage" for the intracoronary infusion after STEMI in their study (Meluzin et al, 2006). Although some other studies did not found such relationship (TOPCARE, REGENT), improvement of regional LV function was "cell-dose" dependant in this study. Regeneration by Intracoronary Infusion of Selected Population of Stem Cell in Acute Myocardial Infarction (REGENT) trial (Tendera et al, 2009) is important for two reasons. The first is the patients' selection, with the enrollment of patients with more severe LVEF impairment (LVEF≤40%) and the second is the immunomagnetic selection of bone marrow MNC for CD34+/CXC4R+ cells which represents the "selection" arm in this study. Unfortunately MRI follow-up was paired in only 59% of patients. Again, patients with baseline LVEF less than median had the significant improvement of LVEF after 6 months in both cell groups (selected and non-selected). However, the median baseline LVEF value in this study was 37%, meaning that a half of patients have had the baseline LVEF between 37-40%, probably indicating the recruitment bias in this study. The FIN study of autologous bone marrow-derived stem CELLs in acute myocardial infarction (FINCELL) for the first time used intracoronary stem cell therapy a few days after successful thrombolysis (Huikuri et al, 2008). The intracoronary injections of bone marrow MNC were given immediately after percutaneous coronary intervention which was performed on the already opened infarct related artery. Intracoronary injections of stem cells in these patients were feasible and associated with the improvement of LVEF after 6-months. Meticulous assessment of arrhythmogenic potential of stem cells was done in this study using three non-invasive methods (Holter monitoring, microvolt T wave alternans and Signal-averaged electrocardiogram) having proved that intracoronary bone marrow cell therapy did not seriously aggravate arrhythmias. Intravascular ultrasound imaging performed in this study confirmed that cell therapy did not cause restenosis. The HEBE trial (Hirsch et al, 2010) investigated the influence of bone marrow compared to peripheral blood derived MNC intracoronary and controls to global and regional LV function measured by MRI. This relatively large trial resulted in neutral influence of cell therapy on LV performance after 6 months. The relatively short ischemia time in this trial may explain the equal and significant recovery of LVEF in all three arms of this trial. Besides, the baseline LVEF was above the 40% (median=43.4%) pointing that the majority of patients in this study had good prognosis and no additional benefit of stem cell therapy should be expected. Indeed, there was a trend toward better results of stem cell therapy according to percent of the regional segment improvement in patients with baseline LVEF bellow the median value. The French study (Roncalli et al, 2010) was concentrated to the scintigraphy analysis of viability after intracoronary infusion of bone marrow derived MNC. Patients with more severe infarction (LVEF≤45%) were enrolled in this study. Bone marrow cells slightly improved viability in cell therapy group. This study also emphasized the negative impact of smoking on the improvement of viability during time.

Only three trials published their long-term results of intracoronary bone marrow derived cell therapy in the acute phase of STEMI. Strauer's group, in their non-randomized, but well controlled study had showed that the benefit on intracoronary bone marrow derived MNC infusion after infarction for the myocardial performance sustained after 5 years and that even decreased the abnormal heart rate variability, late potentials and ectopic beats (Yousef

et al, 2009). And the most important, mortality of BMC-treated patients was significantly reduced in comparison with the control group. The long-term study of Chinese group (Cao et al, 2009), also indicated the persistent improvement of LVEF (over 4 years) in AMI patients treated with intracoronary bone marrow MNC compared to controls, but interestingly without significant improvement on viability. In BOOST trial (Meyer et al, 2009) patients with more transmural extension of infarction appeared to benefit from BMC transfer throughout the five years.

Most likely, single intracoronary cell infusion cannot bring enough stem cells into the infarction area for the sustained beneficial effect on the myocardial function. There is probably the saturation level of stem cell delivery in such short period of time which precludes their significant influence on myocardial regeneration in patients with very large myocardial necrosis. Yao's group, in their relatively small study suggested that repeated intracoronary stem cell therapy, after 3-7 days from STEMI and again after 3 months may have an additional advantage in comparison to single early stem cell treatment (Yao et al, 2009).

The extraordinary trial comes from the Hare's group, who for the first time used intravenous allogeneic mesenchymal stem cells infusion from the healthy unrelated bone marrow donor in patients with STEMI (Hare et al, 2009). Mesenchymal stem cells lack major histocompatibility complex and costimulatory cell-surface antigens which enable their allogeneic transfer and secret various anti-inflammatory cytokines promoting healing. They are also rich in the homing properties which allow intravenous application. This study performed detailed safety assessment including pulmonary function and computed tomography of chest abdomen and pelvis in the follow-up. Mesencymal stem cell therapy demonstrated reduced ventricular tachycardia, better pulmonary function and increase of LVEF in patients with anterior infarction compared to controls.

Two trials examine the safety, feasibility and efficacy of trans-endocardial route of bone marrow derived MNC delivery using electromechanical mapping as the guidance (NOGA system) after AMI. MYSTAR trial (Gyöngyösi et al, 2009) compared early (3 weeks after AMI) and late (3 months after AMI) combined trans-endocardial and intracoronary bone marrow derived MNC. In both arms cell therapy achieved small but significant improvement of LVEF measured by g-SPECT. This study used a large number of CD34+ cells, and the majority of cells were given intracoronary. Unfortunately this study had no arms with intracoronary and trans-endocardial route of delivery separately and we do not know if the combined route of stem cell delivery has any synergistic effect. Krause et al, published their small, uncontrolled study with early trans-endocardial delivery of bone marrow MNC in AMI, and they proved its safety with the significant improvement of LVEF after six months (Krause et al, 2009).

Several studies (Table 2) investigated the usage of granulocyte growth factor (G-CSF) for induction of longer and increased mobilization of stem cells during the first days of AMI (Valgimigli et al, 2008). The application of G-CSF for several days achieved the 10-30 times, increased of CD34+ cells number in peripheral blood (Ince et al, 2005; Valgimigli et al, 2005; Zohlnhöfer et al, 2006; Engelman et al, 2006; Ripa et al, 2006; Takano et al, 2007; Leone et al, 2007). When we analyzed the results of these studies it seemed that very early start of G-CSF after STEMI (during the first day) and its application in patients with lower LVEF (lower than 40%) had a positive effect on systolic function (Ince et al, 2005; Takano et al, 2007; Leone et al, 2007). However, G-CSF had some potential prothrombotic and pro-inflammatory effects (Le Blanc et al, 1999; Falanga et al, 1999) which could be deleterious for

patients with AMI, but it was not seen in the current published trials. Parathyroid hormone or its analogs may be an alternative drug for stem cell mobilization in this setting (Huber et al, 2010).

Study	The number of patients	Time GCSF	Duration of G-CSF therapy and dosage	Patient selection	Results of the study
FIRSTLINE-AMI	25GCSF/10 C	1.5 h-pPCI	6d, 10 µg/kg/d s.c.	1st-AIM, pPCI	ECHO-EF I WMSI↑, PET ↑
STEMMI	39 GCSF/39C	2 d	6d, 10 µg/kg/d s.c.	1st AIM, pPCI	MRI wall thick, EF↔,
G-CSF-STEMI	23 GCSF/21C	2 d	5 d, 10 µg/kg/d sc	1st AIM, pPCI	MRI-EF, vol. and reg. function↔, perfusion↑
REVIVAL-2	58 GCSF/56C	5 d	5d, 10 µg/kg/d s.c.	1st AIM-lysis, PCI 5d	SPECT IS ↔, MRI-EF↔
REGENERA	14 GCSF/27C	≥5 d	5d,10 µg/kg/d s.c.	1stant AIM EF<50%	ECHO-EF, vol. and WMSI↑
TAKANO	22 GCSF/18C	1 d	5d,2.5 µg/kg/d s.c.	1st ant AIM pPCI	gSPECT-EF, vol. IS↑

Table 2. Important clinical trials used mobilization of stem cells to treat acute myocardial infarction.GCSF- Granulocyte colony-stimulating factor, AIM - ST elevation myocardial infarction, pPCI- primary percutaneous coronary intervention, dECHO- dobutamine echocardiography, EF- ejection fraction, WMSI- wall motion score index, EDV- end-diastolic volume, gSPECT- gated single-photon emission computed tomography, PET- positron emission tomography, MRI- magnetic resonance imaging

3. Important clinical trials on stem cell therapy in chronic myocardial infarction

The chronic myocardial infarction (CMI) represents a completely different environment for the stem cell therapy. The precise definition of chronic is not established, but it seems that it would be accepted that the chronic MI may be old at least 1-2 months after the necrotic event. Highly dynamic inflammatory reaction with cellular and cytokine storm is finished and slow fibrotic process replaces it (Frangogiannis, 2008). The abundance of chemokines, growth factors, adhesion molecules and other biologically active substances in the acute inflammatory phase of infarction not longer exist. Some parts of myocardium adjacent to infarction core due to long time of ischemia and because of partly damaged structure after the index event are alive but not capable for fully function. Those areas need revitalization with stem cells, but the question is whether the same cells are needed for the chronic IM as for the acute MI, and whether the same route of delivery would be equally efficient? Very interested human pilot study of tracking the labeled circulating progenitor cells (CPC) with indium oxine (^{111} In-oxine) after intracoronary injections in patients with acute (<15 days), intermediate phase (15 days-1 year) and a late chronic stage of MI (>1 year), demonstrated that amount of progenitor cells retained in the

myocardium decreased progressively over the time (Schächinger et al, 2008) alludes the answer on the second question. Human trials comparing bone marrow derived MNC and peripheral blood progenitor cells (PBPC) exist at least for AMI patients with inconclusive and contradictory results on their regenerative capacity (Schächinger et al, 2004; Hirsch et al, 2010). However, those cells are very similar but the only difference is that bone marrow MNC cells have more primitive cell subpopulation then PBPC which are more commitment to endothelial lineage. The comparison of mesenchymal stem cells and hematopoietic CD34+ cells in animal model of myocardial infarction showed that mesenchymal stem cells were more potent for the healing of the heart (Arminan et al, 2010).

Method of SC delivery Study	**Number of patients In groups**	**Timing of SC therapy after MI**	**Bone marrow volume, the number of cells**	**Selection of the patients**	**The main results of the study**
I.C.					
TOPCA RE-CHD	28 BMMNC/24 CPC/23 C	>3 m	50 ml BM, 270 m PB, Ficoll, 2.0±1x10^{6}MNC/ 22±11x10^{6}CPC	Patent IRA	LVA, MRI, PET - EF↑, regional function↑ - BMMNC
STAR	191 BMMNC/200 C	8.5±3.2 y	80-120 ml, Ficoll, 6.6x10^{7}BMC	Patent IRA by PCI, EF≤35%	LVA, EF and regional function↑, exercise capacity↑, Mortality↓
MAGIC-DES	25 BMMNC-AMI/25 AMI-C/ 16 BMMNC-CMI/16 CMI-C	≤14 d, 2±3 d-AIM/>14 d-CMI ≈2 y	GCSF s.c.10μg/kg 3d, 4 d COBE-BCT, 1.4x10^9 Leu, CD34-9.2±10.4%	Patent IRA	MRI-EF ↑ in AMI CPC group, in CMI ↔
I.C. vs. I.M.					
Ang et al	21 BMMNC IC/ 21 BMMNC IM/ 20 C	>6 w	80 ml, Lymphoprep, 1.4x10^5 CD34+ I.M./2.4x10^5 CD34+ I.C.	Graftable infarct area	d-ECHO, MRI – EF and regional function↔ in all groups
Epicardial					
Patel	10 BMMNC/ 10 c	NS	550 ml, Ficoll, immuno-magnetic sel. 22x10^{6}CD34+	Graftable infract area, EF≤35%	ECHO, gSPECT – EF↑
Mocini	18 BMMNC/18 C	Recent MI >4 w and < 6 m	50 ml, seeded with HES, centrifugation, 3.7x10^{9}CD34+ after CABG during arrest	Graftable infract area, LVEF≥35%	MRI – EF↑ and WMSI↓

Method of SC delivery Study	Number of patients In groups	Timing of SC therapy after MI	Bone marrow volume, the number of cells	Selection of the patients	The main results of the study
Hendrikx	10 BMMNC/10 C	217±162 d	40 ml, Lymphoprep, 60.2x10^6 BMC, CD34% 1.4±1.0	Graftable infract area	MRI - EF↔,, SPECT – viability ↔
Stamm	20 BMMNC/20 C	7-9 w	90-250 ml, immune sel. 133+/CD34+ 6.0x10^6	Graftable infract area	ECHO EF↑, SPECT-viability↑
Zhao	18 BMMNC/18 C	18-21±17 m	30 ml, Ficoll, 6.6x10^8 BMMNC	Graftable infract area, EF<40%	ECHO - EF↑, volumes↓, regional function ↑, SPECT↑
MAGIC	30 HDMy/33 LDMy/34 C	>4 w	10 g of tigh muscle, 3 w of culturing, HDMy-800x10^6, LDMy-400x10^6	Graftable 15%≥EF ≤35%	ECHO - EF↔, ESV↓ in HDMy group
Endocardial					
Perin	14 BMMNC/7 C	>3 m	50 ml, Ficoll, 5.7±6.1x10^{4}CD34+	Ineligible for revasc. EF<40%	LVA - EF↑, EMM - viability↑
Pokushalov	55 BMMNC/54 C	>12 m, 9±8 y	NS, Ficoll, 1.0±0.6x10^{6}CD34+	Ineligible for revasc.EF< 45%	ECHO - EF↑, SPECT - viability↑, functional status↑, 6 min WT↑
SEISMIC	26 My/14 C	8 y IQR 4-12	10 g of tigh muscle, 2-3 w of culturing, My-100-400x10^6	Ischemic HF	RNV-MUGA - EF↔, functional status trend↑
Ramshorst	25 BMMNC/25 C	NS	80 ml, Ficoll, 40x10^{6}MNC	Ineligible for revasc. EF<40%	SPECT↑, MRI - EF↑

I.C.- intracoronary, I.M. intramyocardial- during CABG, BMC-Bone marrow cells, BM-MNC – Bone marrow mononuclear cells, CPC-circulating progenitor cells, EPC- endothelial progenitor cells, My-Myoblasts, HD- high dose, LD- low dose, Bone marrow mesenchymal stem cells, PB-peripheral blood, C-controls, IQR- interquartile range, CABG- coronary artery bypass grafting, revac.- revascularization, HF- heart failure, dECHO- dobutamine echocardiography, gSPECT- gated single-photon emission computed tomography, PET- positron emission tomography, MRI- magnetic resonance imaging, RNV-radionuclide ventriculography, 6 min WT- six minutes walking test, LVA- left ventricle angiography, EMM- electro-mechanical-mapping, IVUS- intravascular ultrasound, MLA- minimal lumen area, EF-ejection fraction.

Table 3. Important clinical trials of stem cell therapy for CMI.

Clinical trials of stem cell therapy in CMI (Table 3) are smaller and not so well conducted as trials of stem cell therapy in AMI (Sanz-Ruiz et al, 2010; Donndorf et al, 2011). According to coronary status we can divide patients with CMI in two groups, the first one eligible for revascularization of the infracted area and the second with no option of revascularization. We believe that it is very important to perform as complete as possible revascularization before the stem cell therapy and not to proceed to sophisticated stem cell trial in ischemic cardiomyopathy without knowing the coronary status of enrolled patients (C-CURE, NCT00810238). Again, the different modes of stem cells and methods of delivery might be necessary in those two groups. Based on some animal models (Hou et al, 2005) and on the logical assumption the direct intramyocardial (trans-epicardial in patients who need surgical revascularization and trans-endocardial in patients who have no option of revascularization) route of stem cell delivery might be a preferred option.

Transplantation of Progenitor Cells and Recovery of LV Function in Patients with Chronic Myocardial Infarction (TOPCARE-CHD) was the first randomized, cross-over study examining the role of intracoronary bone marrow stem cell therapy for CMI (Assmus et al, 2006). The transplantation of bone marrow derived MNC was associated with the modest but significant improvement of six-months LVEF (ΔLVEF=4.8% measured by MRI) and regional myocardial function. The improvement of the functional status assessed by the NYHA classification was also significant in the BMMNC group. The second large, not randomized but well controlled study was Stem cell Transplantation in 191 patients with chronic heart failure - STAR-heart study (Strauer et al, 2010).

The intracoronary injections of BMMNC had sustained (3 months – 5 years) beneficial effect on LV global and regional function, increased exercise capacity, improved functional capacity and reduced mortality compared to controls. Myocardial Regeneration and Angiogenesis in Myocardial Infarction study (MAGIC-DES) compared the influence of intracoronary injections of G-CSF mobilized PBPC on LV performance between patients in AMI and CMI previously treated with drug-eluting stents (Kang et al, 2006). Only patients with AMI had improvement of LVEF after 6 months. The study of Ang, compared intra coronary (through the graft) and intramyocardial injection of bone marrow derived MNC and controls during CABG (Ang et al, 2006). Stress echocardiography did not reveal any improvement in viability in the akinetic segment. MRI follow-up was done in only one third of patients in this study.

The first small study of application bone marrow derived MNC into the myocardium was the study of Hamano (Hamano et al, 2001). They injected bone marrow MNC into the non-graftable area during the coronary artery bypass grafting (CABG) and found that the procedure was feasible, safe and that induced improvement of myocardial perfusion assessed by SPECT in 3/5 patients. The detailed description of patients was not presented.

Patel conducted the first randomized trial with intramyocardial injections of enriched suspension of CD34+ cells during the off-pump CABG in patients with severe ischemic cardiomyopathy (Patel et al, 2005). Intramyocardial bone marrow derived MNC transplantation with off-pump CABG led to significant improvement of 6 months LVEF and functional status compared to patients treated with surgery only.

In the randomized trial of Mocini injections of bone marrow MNC into the peri-infarcted and infracted region (only patients with recent infarction were included) after the CABG during the cardiac arrest was compared to CABG alone (Mocini et al, 2006). The patient cohorts had moderate LV systolic dysfunction (inclusion criteria was baseline EF>35%).

There was no increase of serious arrhythmias. Transplanted patients had significant improvement of EF and WMSI measured by MRI after 6 months compared to the controls.
The relatively small randomized study of Hendrikx demonstrated only improvement of regional, but not the global systolic function by 6-months MRI follow-up with the bone marrow MNC myocardial injections after CABG (Hendikx et al, 2006). Interestingly the number of CD34+ cells injected was significantly higher in the responder group what implied the possible importance of cell dosing.
The randomized study of Stamm, investigated the influence of more premature CD133+ cell myocardial injections after CABG on myocardial function and perfusion (Stamm et al, 2007). The significant improvement of EF and myocardial viability was detected after 6 months in the cell therapy group. Subgroup analysis showed that patients with the lower EF had the greater benefit for selected stem cell therapy. The injection of selected more premature cells was safe.
The study of Zhao corroborated with the previous investigations, and verified the benefit of intramyocardial injections of MNC during CABG in patients with severe impaired EF post-infarction on global and regional myocardial function and perfusion (Zhao et al, 2008).
Very interesting non-randomized, case control study comes from Thailand's group, who used thoracoscopic delivery of in-vitro expanded endothelial progenitors (EPC) isolated from the peripheral blood into the peri-infarction area (Arom et al, 2008). The subset of patients received combined EPC therapy with off-pump CABG. They enrolled patients with very severe ischemic heart disease and low basal EF (26±7%). EPC transplantation improved significantly LVEF even combined or not with CABG. This study is important because it gives a possible solution for very ill patients with chronic ischemic cardiomyopathy and with no option for revascularization. The procedure is minimally invasive, safe and might help.
The clinical application of stem cell therapy had started with intra-myocardial injection of myoblasts. Menasche reported the first successful case on myoblast implantation during CABG and significant improvement of EF throughout 6 months (Menasche et al, 2001). Seven years later definitive results of Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial were published (Menasce et al, 2008). The study had three arms, high and low-dose myoblast groups and a placebo group. Myoblasts were obtained from thigh biopsy and in vitro cultivation for three weeks. All patients received implantable cardioverter-defibrilator at the time of tight biopsy. Myoblasts were injected neighboring the akinetic segments. The modest increase of EF after 6 months was noticed in all groups equally. Nevertheless, patients who received high number of myoblasts had a significant decrease of end-systolic volume.
Transmyocardial route of stem cell delivery guided with electro-mechanic mapping (NOGA system) represents an alternative option for the treatment of patients ineligible for conventional revascularization. Perin's group conducted the pioneering, non-randomized but controlled study of trans-endocardial bone-narrow MNC injections using the electromechanical mapping (NOGA system) to guide cell injections into the viable but not mechanically functional myocardium (Perin et al, 2003). Patients treated with cell therapy had a greater increase of EF measured by RNA, reduction of reversible defect on SPECT and improved functional status after 2 and 4 months follow-up.
Four relatively larger randomized studies with trans-endocardial application of bone marrow derived MNC or myoblasts have been recently published. Pokushalov's group (Pokushalov et al, 2010) randomized patients with end-staged ischemic cardiomyopathy with chronic MI were assigned to trans-endocardial injections of bone marrow MNC and

the control group. Cell therapy provided the significant improvement in functional status, angina score, myocardial perfusion and global EF in comparison to control arm. Mortality also significantly decreased in cell therapy group (10.9% compared to 38.9%, $p<0.001$). The extremely valuable study comes from Ramshort group. They examined a role of trans-endocardial injections of bone marrow derived MNC into the electrically alive but functionally inactive myocardium in patients with severe, refractory angina and without additional option of revascularization (Ramshort et al, 2009). More than half number of patients had had previous MI in both cell therapy group and control group. Stress-induced ischemia was reduced after 3 months and slight improvement of LVEF was demonstrated with cell therapy. This therapy also significantly improved the clinical status of patients.

Other two studies (SEISMIC and CAuSMIC) implanted cultured autologous myoblasts vie NOGA guiding system in patients with severe ischemic heart disease, previous infarction and chronic heart failure symptoms (Dib et al, 2009; Duckers et al, 2011). A high percentage of patients in both studies had previously ICD implanted. There was favorable safety with no difference between groups in arrhythmias and deaths. In both studies there was a functional improvement in myoblast groups, but SEISMIC study did not show any EF increase, and CAUSMIC sustained reduction of LV diameters.

Two pilot trials with adipose derived stem cells (ADSC), one with intracoronary injections of ADSC in patients with STEMI (A Randomized Clinical Trial of Adipose-Derived Stem cells in the Treatment of Patients With ST-Elevation myocardial Infarction - The APOLLO Trial) and one with intra-myocardial injections of ADPC in patients with severe ischemic heart disease and illegible for revascularization (adipose-derived stem & Regenerative Cells In the Treatment of Patients With Non revascularizable ischemic Myocardium - The PRECISE Trial) showed feasibility, safety and initial promising results.

4. Our experience

Forty two patients enrolled in the REANIMA study **(Regeneration of myocardium with bone marrow mononuclear cells in myocardial infarction)** underwent the autologous, bone marrow derived stem cell therapy for myocardial infarction in our Institution (Military Medical Academy, Belgrade) in the period from February 2004 to September 2010. The Local Ethical Committee approved the study and the informed written consent was obtained from all participants. All patients reperfused successfully with primary percutaneous coronary intervention or by thrombolytic therapy (accelerated protocol with Actilyse, Boehringer-Ingelheim) between 2-12 hours from the pain onset.

Three groups were formed. Group I received intracoronary injection of bone marrow derived MNC on 6-12 day after MI; group II received intracoronary injection of bone marrow derived MNC in the chronic phase of infarction; and group III received bone marrow derived MNC intramyocardially during the CABG. The inclusion criteria for the first group were the presence of the first MI, age under 70 years, opened infarct related artery on the 5th day of infarction, LVEF≤40% on the 5th day, and the clinically stable patient. The inclusion criteria for the II group were age under 70, MI at least 2 months before stem cell therapy, clinically stable patient, and LVEF≤40%. Finally, the inclusion criteria for the III group were age under 70, indication for CABG, the graftable infarction related area, LVEF≤45%, and the clinically stable patient. The common exclusion criteria were the presence of the important comorbidities (systemic or cardiac).

In AMI group baseline echocardiography assessment was performed between 4-7 days. The LVEF was determined according to the Simpson's rule, wall motion score index at rest and end-diastolic and end-systolic volume indices were measured. Examination was repeated in the sixth month after MI.

Single-photon emission tomography (SPECT) with Technetium 99^{m}-sestamibi was done between 4-7 day and in the 6^{th} month. The infarction size (IS) of left ventricle (LV) was quantified by the commercial software (AutoQuant software, Cedars-Sinai QPS/QGS component of AutoQuant) as an area of LV (in percentage) with the uptake of tracer less than 50% of the maximal value.

Twenty-four hour ECG Holter was done in all patients in the second month from cell therapy.

The harvest of bone marrow was done in the morning of cell therapy. For intracoronary MNC delivery, between 250-350 ml of bone marrow was harvested under the general anesthesia with the multiple aspirations from the posterior iliac crests. After harvesting, cell suspension was filtered twice and processed with the COBE SPECTRA to reduce the number of red cells and platelets. The total final cell suspension volume was 150 ml, and the total cell number was between 10-50x10^{9}/µl. MNC represented 25-40% of these cells, and CD34+ cells were between 1.5-2.0% of it. Cell suspension was given through the diagnostic catheter deeply positioned in the LM. Boluses of 20 ml were given during 1 minute with 2 minutes pauses apart from the injections. Transient ST segment elevation was noticed in every patient. A slight increase of troponin was detected in one patient in CMI and one in AMI group after the procedure with minimally prolonged chest pain.

The bone marrow harvest (150 ml) for intramyocardial cell transfer was done under the general anesthesia immediately before the CABG. Cells were processed manually and after several filtration and centrifugation steps total volume of 15-20 ml was prepared. Preparation of cells was done during the operation, and cell injections of 20-30x0.3 ml per injection were performed after the end of operation during the cardiac arrest in the myocardial area adjacent to necrotic core. The mean number of intramyocardial injected CD34+ cells was 2.2±1.1x10^{6} cells. Time from the bone marrow harvest to MNC application was 3-4 hours in all three groups.

Charcteristics	Intracoronary BMMNC in AMI (N=19)	Intracoronary BMMNC in CMI (N=9)	Intramyocard.BMMNC CMI-CABG (N=14)	P
Age - y±SD	50±11	50±12	54±11	NS
Gender - n (%)				
Female	3 (15.8)	2 (22.2)	0 (0.0)	NS
Risk factors				
Diabetes – n (%)	2 (10.5)	1 (11.1)	3 (21.4)	NS
Hypertension – n (%)	8 (42.1)	3 (33.3)	7 (50.0)	NS
Active smoking – n (%)	13 (68.4)	5 (55.6)	5 (35.7)	NS
Hypercholesterolemia – n (%)	12 (63.2)	5 (55.6)	8 (57.1)	NS
Infarct related artery – n (%)				
LAD	18 (94.7)	9 (100.0)	11 (78.6)	NS
LCX	1 (5.3)	-	1 (7.1)	
RCA	-	-	2 (14.3)	

Table 4. Baseline demographic data of study patients. BMMNC- bone marrow mononuclear cells, AMI- acute myocardial infarction, CMI- chronic myocardial infarction, CABG- coronary artery bypass grafting, LAD- left anterior descending, Left circumflex artery, RCA- right coronary artery.

Baseline demographic characteristics of patients (Table 4) were similar throughout groups. Patients with CMI treated with intracoronary injections of bone marrow MNC had lower LVEF, larger end-diastolic and end-systolic volumes indices and larger infarction size at baseline and after 6 months.

Left ventricle EF significantly increased in patients with intracoronary injections of bone marrow MNC after AMI (ΔLVEF=5.5±6.6%) and in patients treated with intramyocardial injections of bone marrow MNC (ΔLVEF=5.0± 4.2) and there was no change of LVEF in patients with intracoronary injections of bone marrow MNC in CMI (Figure 2). The infarction size was significantly reduced in patients with intracoronary injections of bone marrow MNC after AMI (ΔIS=6.2±5.0%) and in patients treated with intramyocardial injections of bone marrow MNC (ΔIS=4.9± 4.3) and there was no change of infarction size in patients with intracoronary injections of bone marrow MNC in CMI (Figure 2).

End-points	Intracoronary BMMNC in AMI N=19	Intracoronary BMMNCin CMI N=9	Intramyocardial BMMNC in CMI after CABG N=14	P value between 3 groups
Baseline LVEF (%)	33.1±4.1	30.8±4.4	35.3±3.9	0.05
6-m LVEF (%)	38.6±8.3	29.9±6.53	40.3±5.4	0.01
ΔLVEF %	5.5±6.6	-0.9±2.7	5.0±4.2	0.01
	P =0.002	P=0.354	P=0.001	
Baseline EDVCI ml/m²	68.2±11.3	90.8±29.3	70.3±22.5	0.02
6-m EDVCI ml/m²	72.5±12.8	94.2±35.1	70.7±15.3	0.02
ΔEDVCI ml/m²	-4.4±10.1	-3.5±12.4	-0.4±13.2	0.63
	P=0.080	P=0.428	P=0.920	
Baseline ESVCI ml/m²	44.1±9.9	63.4±23.7	48.4±15.3	0.01
6-m ESVCI ml/m²	44.5±11.0	65.3±30.3	42.1±10.9	0.01
ΔESVCI ml/m²	-0.3±7.8	-1.9±9.6	6.3±11.0	0.07
	P=0.852	P=0.575	P=0.050	
Baseline IS (%)	30.3±8.5	37.9±9.1	28.9±4.1	0.19
6-m IS (%)	25.3±11.0	37.4±8.4	22.7±5.2	0.01
ΔIS	4.9±4.3	0.4±1.4	6.2±5.0	0.02
	P<0.001	P=0.377	P<0.001	

Table 5. Left ventricle ejection fraction (LVEF) and infarction size (IS) at baseline and after 6 months.

Although improved LVEF, intracoronary bone marrow MNC transfer in patients with AMI did not block remodeling of the left ventricle. There was a trend toward significant increase of LV end-diastolic volume index in those patients (Table 5). On the other side, patients treated with intramyocardial bone marrow MNC injections with CABG had a positive effect on end-systolic volume index which significantly decreased after 6 months. In patients with CMI, there were no significant changes of either LVEF, or volume indices, or IS after six months (Table 5).

After six months of follow up, there were no deaths in any group (Table 6). Other important clinical event is showed in the table 6. We did not observe any significant arrhythmias on 24

hours ECG Holter during the follow-up of six months. Patients with CABG and cell therapy were the most stable. Also, functional NYHA class in six months was lower in CABG plus cell therapy treated patients compared to other two groups.

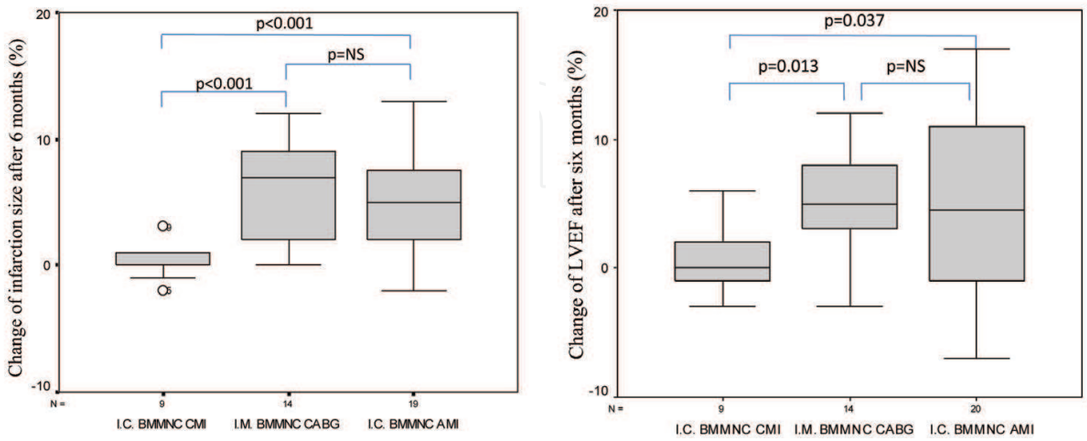

Fig. 2. Changes of LVEF and IS between 6-months and baseline measurements across the three groups. I.C. BMMNC-CMI- Intracoronary bone marrow mononuclear cells in chronic myocardial infarction; I.M.BMMNC-CABG- intramyocardial bone marrow mononuclear cells in chronic myocardial infarction after coronary artery bypass grafting; I.C.BMMNC AIM- intracoronary bone marrow mononuclear cells in acute myocardial infarction

Major adverse cardiac events	Intracoronary BMMNC in AMI (N=19)	Intracoronary BMMNC in CMI (N=19)	Intramyocardial BMMNC in CMI after CABG (N=14)
Revascularization n (%)	4 (21.1)	1 (11.1)	-
Heart failure – n (%)	3 (15.8)	4 (44.4)	1 (7.1)
NYHA class 6 months	1.58	1.14	1.89

Table 6. Major cardiac adverse events after 6 month follow-up.BMMNC- bone marrow mononuclear cells, AMI - acute myocardial infarction, CMI - chronic myocardial infarction, CABG - coronary artery bypass grafting

Our study is small and non-randomized, but nevertheless, suggests two important conclusions. The first is that bone marrow derived, native stem cells showed the improvement of the left ventricle function and a decrease of infarction size in both patients with AMI and CMI, and the second, direct intramyocardial delivery of bone marrow derived MNC is probably more efficient than intracoronary route of administration in patients with CMI. In our previous study (Obradovic et al, 2009a, 2009b) we compared function of LV and reduction of infarction size in patients with acute myocardial infarction treated with intracoronary bone marrow cell injections to well-matched control group and showed trend of improvement of LVEF and significant reduction in infarction size in cell therapy group. The improvement of LVEF by 5% in our trial of AMI patients is in accordance with the results of REPAIR AMI trial (Schachinger et al, 2006a, 2006b) and the result of meta-analysis of intracoronary bone marrow derived stem cell transplantation in AMI patients (Abdel-Latif et al, 2007).

The outcome of stem cell therapy depends on different factors. The proper selection of patients, timing and methodology of stem cell therapy is crucial for improvement. In AMI we have a reasonable assumption that patients with lower LVEF had increased benefit of bone marrow derived stem cell therapy. However, among larger trials with intracoronary bone marrow derived stem cell therapy for AMI, only REGENT trial (Tendera et al, 2009) have had the entrance criteria of LVEF<40%, but it has not been stated when and how LVEF was measured, because it is not equal if it is measured on admission, or 2-3 days after the reperfusion therapy, and it is difficult to explain how the median of LVEF in this study was 37% with the such entrance criteria for LVEF. This implies some recruitment bias. The entrance echocardiogram in our study was performed on the 4-5 days after AMI to avoid myocardial stunning which is very pronounced in the first few days of AMI, and we suggest that entrance LV performance should be measured on the 3rd-4th day after AMI and not on admission or within the first 48 hours.

But is there a lower border of infarction damage when the stem cell therapy has no benefit? In our study (Obradovic et al, 2009) we showed that patients with too large myocardial infarction (measured by the perfusion defect on SPECT and by the maximum serum lactate dehydrogenase activity during the acute phase of STEMI) have no benefit of single, intracoronary stem cell therapy. Those patients might need repeated stem cell injections like in Chinese study (Yao et al, 2009) with the repeated intracoronary bone marrow cell transplantation three months after the AMI with the similar cohort of patients as ours.

It seems that intracoronary bone marrow stem cell therapy in early days of stem cell therapy also has no effect (Zhang et al, 2009), because the stem cells are injected in a very hostile, inflammatory, ischemic environment full of toxins. But, there are no randomized trials comparing stem cell therapy, for instance between 1-5 days to 6-12 days after infarction. Like in the most studies with intracoronary transplantation of stem cells in AMI, we injected cells intracoronary in the second week of infarction, not too soon from the initial event and not too late from it, to be in the burst of reparation process.

However, MYSTAR trial (Gyöngyösi et al, 2009) demonstrated that stem cell therapy after 3 weeks and 3 months had resulted in similar benefit on LV function. Having in mind that finding, our experience and previous reports we can only conclude that we do not know the proper timing for stem cell therapy after AMI.

What kind of cells we need in AMI, and do we need another cell type or types for CMI? In our study we only use the filtration of bone marrow and concentration of their mononuclear cells. We suppose that different kind of cells and their interplay is important for the successful cell therapy in AMI. The immune selection of bone marrow stem cells without some in-vitro manipulation of cells is probably unnecessary, especially in AMI patients. What do we gain and what do we lose with this procedure? The same number of cells with certain phenotype would be given with, or without selection, and a selection procedure would for sure prolong the timing from the bone marrow harvest to its application and further damage. The preparation of cells is important, however, at least for patients with AMI it is more important to give appropriate number of viable and functioning cells and the duration of bone marrow processing should be short. In the REGENT trial, immune-selection of CD-34+/CXC4R+ cells did not bring any advantage compared to un-selected bone marrow mononuclear cells.

Again there is no clinical randomized trial comparing different methods of stem cell processing. Do we need mesenchymal stem cells for AMI or CMI patients? Very well

conducted study (Chen et al, 2004), with successful intracoronary implantation of mesenchymal stem cells in patients with AMI is almost neglected and those results are not challenged.

The way of cell delivery is also a matter of controversy. For intracoronary delivery almost all studies have used the same method (Strauer et al, 2002) nevertheless, the animal model suggests that the injections of cells through the inflated balloon currently applied in clinical studies are not necessary for cell deposit (Tossios P, et al, 2008). So, our study also has showed that non-selective injections of bone marrow MNC into the left coronary artery proved to be efficient in improving the LVEF and diminishing the infarction size. There is no human trial addresses for that issue. There are also numerous tips and tricks for stem cell delivery that might be important. Strauer used albumin-microaggregates to ensure prolonged passage of stem cells through the infracted microcirculation, and dobutamine infusion (Strauer et al, 2010) to increase the demand of oxygen in myocardium and probably to enhance engratment of stem cells with such treatment. However, does the freshly infracted myocardium need such an ischemic push? We have noticed very clear ischemic changes on electrocardiography monitoring in every patient during the delivery of cell suspension.

Intracoronary way of cell delivery is probably more suitable for the AMI patients because it enables homogenous spread of stem cells throughout the infracted microcirculation full of chemoattractants. On the other hand, a direct intramyocardial injection of stem cells in patients with CMI seems to be preferred mode of cell delivery. Some animal model and pilot human trial confirm this assumption (Hou et al, 2005; Schächinger et al, 2008). Our results have shown benefit of bone marrow derived stem cells given into the myocardium during CABG improving LVEF and myocardial perfusion which is in accordance with other studies of bone marrow derived stem cell therapy with CABG (Donndorf et al, 2011). On the other hand, our results have not shown any benefit of intracoronary transplantation of bone marrow stem cells in patients with CMI. There are only 2 published studies with intracoronary transplantation of bone marrow derived mononuclear cells in CMI and the both of them demonstrated improvement of LV performance after the procedure (Assmus et al, 2006; Strauer et al, 2009). The way of trans-balloon application of stem cells was used in both studies and on the contrary we used non-selective intracoronary implantation of stem cells. This distinction might have the different outcome between our and the mentioned studies and underlines the importance of ischemic preconditioning in this cohort of patients.

Finaly, and probably the most important aspect of stem cell therapy is a clinical benefit. REPEAR-AMI (Schächinger et al, 2010), studies of Strauer's group in AMI (Yousef et al, 2009) and CMI (Strauer et al, 2010) and the largest study with endocardial implantation of bone marrow derived stem cells in CMI (Pokushalov et al, 2010) have showed clear clinical benefit with hard end points during the relatively long period of follow-up. In our study, we have not a sufficient number of patients to show the difference of major adverse cardiac events in several groups of our patients. Nevertheless, there were no deaths during the 6 months follow-up, and the number of patients with restenosis and symptomatic heart failure was low.

When we take into account the benefit of stem cell therapy in the treatment of myocardial infarction one scenario is possible. Stem cells do not improve significantly global or even regional myocardial infarction after MI but do stabilize myocardium on the molecular level with the long-term clinically important benefits through yet unknown mechanisms.

As you can easily realize, there are too many confounding, important factors. It is impossible to randomize all the possibilities. Logic is important but it does not mean that it

is always right. Clinical trials in stem cell therapy are being done too fast, and many trials did not meet the entrance criteria of sample size for the right statistical power. The European Task Force for stem cell therapy in cardiovascular diseases does not recommend the stem cell therapy in wider clinical practice and recommends large, placebo controlled trials (Bartunek et al, 2006). However, do we know enough to create the proper, large clinical trial for stem cell therapy? We believe that centrally coordinated, well-organized, small, always multicentric, pilot trials that address the various issues of stem cell therapy must precede the creation of a large randomized trial.

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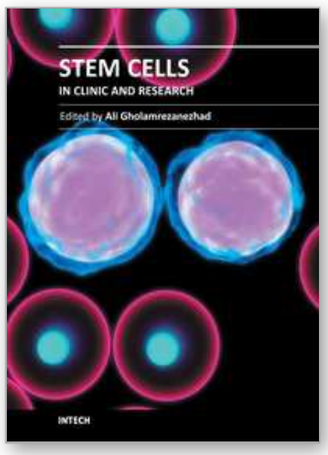

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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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