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**Cell Therapy in Patients with Left Ventricular Dysfunction Due to Myocardial Infarction**

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*Objectives: The purpose of this study was to determine the impact of autologous transplantation of mononuclear bone marrow cells on myocardial function in patients with left ventricular (LV) dysfunction due to an acute myocardial infarction. Methods: The randomized study included 82 patients with a first acute myocardial infarction treated with a stent implantation. This presentation is a subanalysis of 47 patients with left ventricular dysfunction–EF (ejection fraction)* ≤ *40%. Group H patients (n* = *17) received higher number (100,000,000) of cells; Group L patients (n* = *13) received lower number (10,000,000) of cells. The patients of control Group C (n* = *17) were not treated with cells. The Doppler tissue imaging and single photon emission computed tomography were performed before cell transplantation and 3 months later. Results: At 3 months of follow-up, the baseline EF of 35%, 36%, 35% in Groups H, L, and C increased by 6% (P < 0.01 vs. baseline), 5% (P < 0.01 vs. baseline), and 4% (P* = *NS vs. baseline), respectively, as assessed by single photon emission computed tomography (P* = *NS between groups). The baseline number of akinetic segments of 6.9, 7.0, and 6.2 in H, L, and C groups decreased by 1.7 (P < 0.01 vs. baseline), 1.5 (P < 0.01 vs. baseline), and 0.7 (P* = *NS vs. baseline, P* = *NS between groups), respectively, as demonstrated by echocardiography. Conclusion: In our study, the statistically important effect of transplantation of mononuclear bone marrow cells on myocardial function was not found. Only an insignificant trend toward the improvement of global LV EF fraction was found at 3-month follow-up. (ECHOCARDIOGRAPHY, Volume 25, September 2008)*

*stem cells*, *coronary artery disease*, *left ventricular dysfunction*

Postmyocardial infarction congestive heart failure remains to be a major clinical prob lem, despite advances in the medical and sur

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gical treatment of acute coronary syndromes. Coronary artery disease accounts for approx imately 50% of all cardiovascular deaths and is the leading cause of congestive heart fail ure. The 1-year mortality rate for patients di agnosed with congestive heart failure is about 20%, and from 1994 to 2004, deaths from heart failure increased 28%.1*,*2 Development of heart failure in survivors of acute myocardial infarc tion involves myocyte loss in the area supplied by the infarct-related artery and subsequent formation of noncontractile fibrous tissue. To date, no therapeutic procedure like angio plasty or thrombolytic agents could reverse the

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irreversible myocardial injury completely. The recovery of contractile function after revascu larization occurs only in the areas of hibernat ing myocardium. Heart transplantation may be an option in selected patients, but the donor supply is strictly limited.

Recent experimental and clinical studies sug gest that cell transplantation into damaged my ocardium may have the potential to restore myocardial viability and improve left ventric ular function. Different cell types can be po tentially used for transplantation. To avoid problems with donor availability, immunolog ical rejection, arrhythmias, and ethical prob lems, autologous bone marrow cells appear par ticularly attractive. But in a majority of studies, only patients with almost normal function or only mild dysfunction of the left ventricle were studied.3–12

So the purpose of this study was to determine the impact of autologous transplantation of mononuclear bone marrow cells on myocardial function in patients with moderate-to-severe left ventricular dysfunction.

**Materials and Methods**

*Study Population*

The randomized study included patients with a first acute myocardial infarction treated with coronary angioplasty with a stent implanta tion. Only patients with successful recanaliza tion of the infarct-related artery (TIMI flow grade 3) and the evidence of an irreversible damage of at least two akinetic or dyskinetic myocardial segments identified by dobutamine echocardiography, gated technetium-99 m ses tamibi single photon emission computed to mography, and positron emission tomography (performed in only 73% of patients) were in cluded. The exclusion criteria were: (1) age *>* 70 years; (2) noncardiac disease adversely af fecting prognosis; (3) another cardiac disease except coronary artery disease; (4) coagulopa thy, thrombocytopenia, leucopenia; (5) absence of a significant increase in cardiac enzymes (cre atine kinase over 20 *µ*kat/l or creatine kinase MB over 3 *µ*kat/l or troponin I over 20 *µ*g/l- –normal upper limits in our laboratories are 2.85 *µ*kat/l, 0.42 *µ*kat/l, and 2.0 *µ*g/l, respec tively); (6) patient instability on days 3–7 after MI; and (7) need for coronary revascularization in the future for multivessel disease.

From a total number of 82 patients who com pleted the baseline and 3-month follow-up ex

amination, 66 patients were analyzed in the previously published study.12 This first 66 pa tients were randomized into three arms: (1) a group treated with a higher number of mononu clear bone marrow cells (defined as a mean number of 1 × 108 cells); (2) a group treated with a lower number of cells (defined as a mean number of 1 × 107 cells); and (3) a con trol group not treated with cell transplanta tion. Subsequent 16 patients were randomized into only two arms: higher-dose-treated group and control group. The reason for changing ran domization schema was no significant effect of a lower-dose cells in the previous study. This presentation is a subanalysis of 47 (from all the 82) patients with significant left ventricu lar dysfunction-–ejection fraction (EF) ≤ 40%. Forty-five patients underwent the primary an gioplasty (within 12 hours of chest pain on set)and two patients were treated with angio plasty within the interval from 12 hours to 3 days after symptom onset.

*Study Design*

On day 3–6 after myocardial infarction, rest and dobutamine echocardiography was per formed to evaluate the presence of akinetic or dyskinetic left ventricular segments without any contractile reserve. At the same time color Doppler tissue imaging was performed. Within the next 2 days patients underwent the gated technetium-99 m sestamibi single photon emis sion computed tomography and positron emis sion tomography. Patients with an evidence of an irreversible damage of at least two akinetic or dyskinetic myocardial segments proved by all methods were then randomized. Patients of cell groups underwent subsequently a bone marrow aspiration. Autologous bone marrow mononuclear cells were transplanted into the infarct-related artery 20–21 hours after the bone marrow aspiration, 5–9 days after my ocardial infarction. Immediately before and 10 and 20 hours after the procedure, blood samples for cardiac enzymes (creatine kinase, creatine kinase–MB and troponin I) were acquired.

Three months after randomization, rest echocardiography with Doppler tissue imaging, single photon emission computed tomography, and coronary angiography were repeated. Pa tients of the control group underwent the same procedures and examinations, as did the trans planted patients except for bone marrow aspi ration and cell transplantation.

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In this subanalysis, the changes of following echocardiographic parameters were assessed: (1) the peak systolic velocity of the myocardium adjacent to mitral annulus of infarcted wall (Sinfarct) (as a parameter of the regional longi tudinal left ventricular systolic function); (2) the mean six-site systolic velocity of the my ocardium adjacent to mitral annulus (as a pa rameter of the global longitudinal left ventric ular systolic function), which was calculated as mean six-site S = (Slateral + Sseptal + Santerior + Sinferior + Santeroseptal + Sposterior)/6; and (3) num ber of akinetic segments.

The changes of following parameters derived from single photon emission computed tomog raphy were assessed: (1) left ventricle end diastolic volume; (2) left ventricle end-systolic volume; (3) left ventricle ejection fraction; and (4) perfusion defect size.

The institutional ethics committee approved the study and written consent was obtained from each patient.

*Echocardiography*

Using commercially available equipment Vivid 7 (GE/Vingmed, Milwaukee, WI, USA) with an M3 S transducer, echocardiographic ex aminations were performed in one center. Two dimensional and color Doppler tissue images of apical views (apical 4- and 2-chamber and api cal long-axis views) were obtained and stored digitally for the subsequent offline quantitative analysis using a software incorporated in Vivid 7 (Echopac 7 version 1.3, GE/Vingmed). The wider-angle sector (60–70 degrees) was used to depict two-dimensional images for wall motion analysis. The narrow angle sector (30–45 de grees) was used to obtain color Doppler tissue images of individual left ventricular walls (sep tum, lateral, inferior, anterior, posterior, and anteroseptal walls) at the high frame rates of 172–234 frames per second.

Dobutamine echocardiography was per formed in all patients with starting dose of 5 *µ*g/kg per min. The dose was increased at 5-minute intervals to 10, and 20 *µ*g/kg per min. The parasternal long-axis and three apical views were digitally stored at rest and at the last minute of all doses of dobutamine for a subsequent wall motion analysis. A 16-segment model was used for regional wall motion analysis.13 The akinetic and dyskinetic segments with no improvement in thickening after any dose of dobutamine were regarded as irreversibly damaged. A good interobserver

and intraobserver variability in scoring dys functional segments (agreement 93% and 96%, respectively) and in determining the contractile reserve (agreement 92% and 95%, respectively) has already been described.14

The regional longitudinal systolic function was evaluated from the color Doppler tis sue imaging.15*,*16 Peak systolic velocities (S) were determined for the basal myocardium of each wall adjacent to the mitral annulus (Slateral, Sseptal, Santerior, Sinferior, Santeroseptal, and Sposterior). The results were obtained as a mean from three consecutive heart cycles. Two expe rienced echocardiographers who were blinded to the patient treatment performed the analy ses. The reproducibility of estimation of S val ues of individual walls was evaluated in our initial 3-month project.12 For all S values, the estimated 95% confidence limits for differences between intraobserver (JM) pairs of measure ment revealed repeated results to vary in a range of ± 10.6% as based on the mean primary values and similarly ± 11.5% for the interob server variability (JM and RP). The sufficient interobserver reproducibility was also proved in applied pairwise ANOVA models: only 4.8% of overall variability could be attributed to the dif ferences among observers and the interobserver effect was unambiguously not significant (P = 0.963).

*Gated Technetium-99 m Sestamibi Single Photon Emission Computed Tomography*

Seven hundred forty MBq technetium-99 m sestamibi was injected at rest. Gated single pho ton emission computed tomography imaging acquisition (64 projections from the 45◦ right anterior oblique projection to the 45◦ left pos terior oblique projection) began 1 hour after sestamibi injection using a 2-detector gamma camera (ecam, Siemens, Erlangen, Germany) equipped with a low-energy, high-resolution parallel-hole collimators. The MIBI uptake was analyzed visually and quantitatively on computer-generated polar maps by an experi enced nuclear cardiologist who was unaware of the patients treatment. Pixels with a ses tamibi activity *>* 2.5 SD below the correspond ing normal mean values were considered ab normal. The computer automatically expressed a perfusion defect as the number of abnormal pixels divided by the total number of left ven tricle pixels × 100 project.17 In the viability analysis, the myocardial region with the maxi mum sestamibi uptake was used as a reference

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region. The tracer uptake in other myocardial regions was then expressed as a percentage of the activity measured in the reference re gion. Nonviable myocardium was defined as that having sestamibi uptake below the thresh old of 50% of the maximum project.18 Gated single photon emission computed tomography rest left ventricular ejection fractions and left ventricular end-diastolic/end-systolic volumes were obtained using automated, commercially available software four-dimensional-MSPECT (University of Michigan, Ann Arbor, MI, USA).

*Positron Emission Tomography*

To assess myocardial viability, F-18- fluorodeoxyglucose-positron emission to mography was performed with a whole-body positron emission tomography scanner (ECAT ACCEL, Siemens, Knoxville, TN, USA). Ac quisition was started 50 minutes after the administration of fluorodeoxyglucose (200–250 MBq intravenously) and images of glucose utilization were acquired for 15–20 minutes in a 3D mode. The metabolic defects were analyzed on computer-generated polar maps. The myocardial fluorodeoxyglucose uptake for each part of the left ventricle was normalized to a myocardial region with the maximum fluorodeoxyglucose uptake. A nonviable my ocardium was defined as that having less than 50% of the maximum fluorodeoxyglucose uptake.18

*Bone Marrow Aspiration and Preparation*

The target volume of bone marrow blood (100 ml for the lower cell dose, 150 ml for the higher cell dose) was obtained from iliac crests under local anesthesia and moderate se dation with midazolam, mixed with 4% human albumin and 5,000 IU of heparin, and cen trifuged (15 minutes, 240 g) to receive buffy coat. Mononuclear cells were collected using density gradient centrifugation of the buffy coat (20 minutes, 1,200 g, Histopaque 1077, Sigma-Aldrich, St. Louis, MO, USA), washed, and resuspended. One hundred twenty-five per cent of the target amount of mononuclear cells was added to the CellGro serum-free medium (CellGenix, Freiburg, Germany) to reach 0.3– 1.0 × 106 cells/ml. After an overnight cultiva tion (37 ◦C, 5% CO2) in a teflon bag (VueLife, CellGenix), 105% of the target number of mononuclear cells was withdrawn, washed, and resuspended in the Hank’s salt solution (Sigma

Aldrich) with 4% human albumin and 1,000 IU of heparin into a total volume of 22 ml.

*Cell Implantation*

Autologous mononuclear bone marrow cells were transplanted 5–9 days after the infarc tion onset using a modification of the method described previously by Strauer et al.19 Cells were implanted intracoronary via a percuta neous transluminal catheter into the infarct related coronary artery. A total of seven balloon inflations at the place of previous stent implan tation lasting for 3 minutes were carried out with 3-minute intervals of balloon deflation. At the beginning of each balloon inflation, 3 ml of cell suspension was slowly injected into the artery. All patients were on daily doses of 75 mg of clopidogrel and 100 mg of aspirin and, in ad dition, a bolus of 100 units/kg of body weight of heparin was administered immediately before the procedure to minimize the risk of throm botic complications.

*Statistical Analysis*

Standard descriptive statistics were used to summarize the sample distribution of in dividual variables (means, standard errors, confidence limits). A univariate *t*-test for two independent samples was applied to compare values of parameters between the groups. A paired *t*-test was applied to compare changes in values prior and after the treatment. All parametric tests were performed with the ver ified assumption of normal distribution

(Shapiro–Wilk’s W-test). Two indepen dent samples were mutually compared on the basis of proved homogeneity of variance (Variance ratio F-test). The correlation analysis was based on Pearson’s correlation coefficient. A P *<* 0.05 was considered statistically significant.

Repeated measures ANOVA model was used to test the results obtained by different ob servers (measured in all patients included in the reproducibility test). The pairwise de sign included overall F-test of the main ef fects (i.e., differences among different ob servers) and then estimation of within-observer variability.

**Results**

This subanalysis contains 47 patients. Thirty of them were treated with mononuclear bone

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**TABLE I**

Characteristics of the Study Population

Control (C) Lower Cell Dose (L) Higher Cell Dose (H)

Parameter Group (n = 17) Group (n = 13) Group (n = 17)

Age (years) 52 (2) 55 (2) 55 (5) Men 15 (88%) 12 (92%) 15 (88%) Hypertension 9 (53%) 5 (39%) 5 (29%) Hyperlipidemia 6 (35%) 9 (69%) 7 (41%) Diabetes mellitus 4 (24%) 1 (8%) 3 (18%) Single-vessel disease 11 (65%) 9 (69%) 13 (76%) Double-vessel disease 6 (35%) 3 (23%) 4 (24%) Triple-vessel disease 0% 1 (8%) 0% IRA: LAD 16 (94%) 12 (92%) 16 (94%) IRA: LCX 0% 0% 0% IRA: RCA 1 (6%) 1 (8%) 1 (6%) Maximum CK (ukat/l) 80.2 (11.1) 80.2 (9.4) 68.9 (7.2) Maximum CK-MB (ukat/l) 7.4 (0.6) 7.6 (0.9) 6.8 (0.7) Time from infarct onset to 507 (240) 263 (53) 484 (192) reperfusion (min)

Time from infarct onset to cell – 7 (0.4) 7 (0.3) transplantation (days)

Dobutamine echo

No. of irreversibly damaged segments 6 (0.7) 7 (0.4) 7 (0.7) Medication on hospital discharge

Aspirin 17 (100%) 13 (100%) 17 (100%) Clopidogrel 15 (88%) 13 (100%) 17 (100%) ACE inhibitor 17 (100%) 13 (100%) 17 (100%) Beta blocker 17 (100%) 13 (100%) 17 (100%) Statin 17 (100%) 13 (100%) 17 (100%)

The values are expressed as the mean supplied by standard error (in parentheses) or number (%) of subjects. ACE = angiotensin-converting enzyme; CK = creatine kinase; echo = echocardiography; IRA = infarct-related artery; LAD = left anterior descending coronary artery; LCX = left circumflex artery; No = number; RCA = right coronary artery.

marrow cell implantation–17 patients in the Group H with higher cell doses, while 13 in the Group L with lower cell doses, and 17 of them served as a control Group C. The base line characteristics are presented in Table I. There were no significant differences among the groups.

*The Effect of Cell Transplantation*

*on Myocardial Function and Left*

*Ventricle Remodeling*

The results of echocardiographic examina tions and single photon emission computed to mography data are demonstrated in Table II.

There was a trend toward the prevention of the left ventricle dilatation (end-diastolic vol ume) and the improvement of the left ventri cle ejection fraction in transplanted patients. Patients of the high-doses group significantly improved the regional systolic function (Sinfarct) after 3-month follow-up. We proved significant

improvement in these parameters (left ventri cle ejection fraction, end-systolic volume, peak systolic velocity of infarcted myocardium and number of akinetic segments) in cell therapy patients, as it is documented through signifi cant results of within-group testing. However, there were no statistically differences among the groups.

The side effects have already been pub lished.12

*Phenotype of Transplanted Cells*

The samples were analyzed from 29 patients (in one patient a small sample size did not allow adequate analysis). The transplanted leuko cytes contained in the mean 43.4% CD3+ cells, 2.9% CD16+ cells, 11.0% CD19+ cells, 0.4% CD33+ cells, and 1.1% CD34+ cells, respec tively. The viability of mononuclear cells was evaluated after the cultivation. In all cases, the viability exceeded 95%.

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**TABLE II**

Comparison of Baseline and 3-Month Follow-Up Echocardiographic and Single Photon Emission Computed Tomography Results for the Treatment and Control Groups

C Group L Group H Group

Mutual Comparison (P-Values)*†*

Parameter (n = 17) (n = 13) (n = 17) C vs. L C vs. H L vs. H

Echocardiography

Mean 6-site S (cm/s) 4.9 (0.2) 5.1 (0.3) 5.2 (0.2) 0.821 0.416 0.594 Baseline 5.2 (0.3) 4.9 (0.3) 5.0 (0.2) 0.485 0.611 0.822 Follow-up 0.3 (0.2) −0.2 (0.3) −0.2 (0.2) 0.298 0.153 0.813 Change*‡* 0.393 0.625 0.193

P-value*‡*

Sinfarct (cm/s)

Baseline 4.5 (0.2) 4.2 (0.3) 4.3 (0.2) 0.691 0.975 0.704 Follow-up 4.8 (0.3) 4.4 (0.3) 4.7 (0.3) 0.283 0.432 0.728 Change*‡* 0.3 (0.2) 0.2 (0.2) 0.4 (0.1) 0.261 0.342 0.215 P-value*‡* 0.153 0.337 0.013

No. of akinetic s∗

Baseline 6.2 (0.6) 7.0 (0.4) 6.9 (0.6) 0.366 0.411 0.889 Follow-up 5.5 (0.7) 5.5 (0.6) 5.2 (0.7) 0.995 0.744 0.768 Change*‡* −0.7 (0.4) −1.5 (0.5) −1.7 (0.5) 0.242 0.128 0.798 P-value*‡* 0.062 *<*0.001 *<*0.001

SPECT

EDV (ml) 171 (9) 176 (12) 178 (13) 0.786 0.677 0.907 Baseline 183 (13) 180 (12) 181 (12) 0.841 0.876 0.957 Follow-up 12 (8) 4 (10) 3 (8) 0.509 0.431 0.941 Change*‡* 0.153 0.696 0.713

P-value*‡*

ESV (ml)

Baseline 112 (7) 112 (9) 117 (10) 0.998 0.674 0.694 Follow-up 115 (11) 106 (9) 107 (9) 0.555 0.572 0.949 Change*‡* 3 (8) −6 (7) −10 (4) 0.402 0.094 0.706 P-value*‡* 0.713 0.408 0.023

LV EF (%)

Baseline 35 (1) 36 (1) 35 (1) 0.343 0.939 0.308 Follow-up 39 (2) 41 (2) 41 (2) 0.284 0.324 0.897 Change*‡* 4 (2) 5 (1) 6 (2) 0.609 0.262 0.589 P-value*‡* 0.062 *<*0.001 *<*0.001

Perfusion defect (%)

Baseline 52 (4) 51 (4) 53 (4) 0.880 0.911 0.799 Follow-up 41 (4) 41 (5) 43 (4) 0.872 0.657 0.800 Change*‡* −11 (3) −10 (2) −10 (2) 0.601 0.537 0.958 P-value*‡ <*0.001 *<*0.001 *<*0.001

The values are expressed as the mean supplied by standard error (in parentheses). ∗Identified as nonviable on pretransplant dobutamine echocardiography. *†*Mutual significance “between groups” tested by *t*-test for two independent samples. *‡*Pairwise calculated “within group” change of values tested by *t*-test for two-paired samples.

EDV = end-diastolic volume; ESV = end-systolic volume; LV EF = left ventricular ejection fraction; m = myocardium; S = peak systolic velocity of basal myocardium adjacent to mitral annulus; *S*infarct = peak systolic velocity of the infarcted wall; Mean 6-site S = (Slateral + Sseptal + Santerior + Sinferior + Santeroseptal + Sposterior)/6; s = segments; SPECT = single photon emission computed tomography; other abbreviations as in Table I.

**Discussion**

*Potential Effect of Cell Therapy*

Bone marrow contains a great number of primitive cells that are able to differentiate into specialized cells, for example into endothe

lial cells or myocytes.20–24 Some of these prim itive cells produce different growth factors,21 for example vascular endothelial growth fac tor, basic fibroblast growth factor, and cytokines with proangiogenetic effect. For these reasons, many experimental studies were performed and

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proved the possibility of cell therapy to improve perfusion or/and function of dysfunctional my ocardium.20–26

Despite numerous unresolved questions con cerning the cell transplantation, these first hopeful experimental studies were immediately followed by clinical trials, mostly in patients with acute myocardial infarction. The num bers of patients included are relatively small. Many of these studies are not randomized. The type and amount of cells that are necessary to implant to really regenerate damaged my ocardium are not known.

At present, we do not know the mechanism of action of the implanted cells in studies that found improvement in myocardial function or perfusion following the cell therapy. Recently, several experimental projects described no or only negligible transdifferentiation of adult stem cells into the myocytes.27–30 The benefit of cell transplantation may be induced by the paracrine stem cell effect.31*,*32

*Studies in Patients with Acute Myocardial Infarctions*

Transplantation of mononuclear bone mar row cells into the region of infarcted my ocardium has been previously suggested as a promising alternative treatment for left ven tricle dysfunction. Nevertheless, the results of randomized studies are controversial.3*,*4*,*7–9 Some of them indicated that patients with the most depressed left ventricular contrac tile function had the greatest improvement in contractile function after intracoronary admin istration of bone marrow cells. For example, REPAIR-AMI,33 so far the largest randomized multicenter “cell study,” showed significantly greater increase in the global left ejection frac tion in the bone marrow cell group (5.5% vs. 3.0% in the control group) at 4 months follow up. Higher impact of cells was found among pa tients with a baseline left ventricle ejection frac tion below the median value (48.9%). In these patients, the absolute increase in ejection frac tion was three times higher that in the placebo group (7.5% as compared with 2.5%; absolute difference: 5.0%). Among patients with a base line ejection fraction above median, the abso lute difference between groups was only 0.3% (4.0% vs. 3.7%). Similar observations were pre viously described in TOPCARE-AMI trial,34 in which baseline left ventricle ejection fraction was the only significant predictor of improve

ment in ejection fraction during the 4-months follow-up.

In the randomized, double-blind, placebo controlled study of Janssens group,35 in 67 pa tients with ST-elevation myocardial infarction treated with coronary intervention, no effect of autologous bone marrow-derived stem cell transfer on left ventricle ejection fraction was found. However, the treatment was associated with a significant reduction in myocardial in farct size and better recovery of regional sys tolic function. The effect of treatment on the probability of improvement in regional function showed a predominant interaction in the most severely affected segments. In addition to that, on positron emission tomography examination, patients with larger myocardial infarction had a greater increase in metabolic activity after cell therapy than after placebo infusion.

On the other hand, BOOST trial 36*,*37 did not describe the inverse relation between baseline left ventricular ejection fraction and absolute improvement of the left ventricle function af ter implantation of the bone marrow cells into the infarcted myocardium. At 6-month follow up, patients in a control group of this study im proved their ejection fraction from 51.3% to 52% (0.7% absolute change), while the bone marrow cell group from 50.0% to 56.7% (6.7% absolute change). The bone marrow cell subgroup pa tients with ejection fraction of the left ventri cle *>* 52% increased their ejection by 8.0%, but patients with ejection fraction ≤ 52% only by 4.5%.

The main limitation of these studies is the fact, that patients with only mild left ventricu lar dysfunction were included.

*Studies in Patients with Moderate-to-Severe Left Ventricular Dysfunction*

There are only few trials studying cell ther apy in patients with moderate-to-severe left ventricular dysfunction and their results are controversial too. Bartunek et al.3 described improvement of the left ventricular perfor mance and increased myocardial perfusion and viability among patients with acute myocar dial infarction treated with stenting and intra coronary administration of CD133+ progenitor cells. The left ventricular ejection fraction in creased from 45.0% to 52.1%.

Controversially, ASTAMI trial 11 did not find any significant difference between 47 patients treated with cell transplantations and 50 pa tients in the control group. The left ventricular

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ejection fraction and end-diastolic volume were assessed by single photon emission computed tomography, echocardiography, and magnetic resonance. Improvement versus baseline val ues was found in both groups, but they did not significantly differ. Results were consistent for all the three methods. No improvement in car diac function was also found in Kuethe et al.38 in their study with five patients with a large acute anterior myocardial infarction and intracoro nary mononuclear bone marrow cell implanta tion.

In our previous study,12 the significant and dose-related improvement was found in the re gional systolic function of the infarcted wall af ter cell transplantation. As compared to con trols, a higher cell dose significantly improved global LV systolic function. Both cell doses pre vented the left ventricle from the dilation, while the end-diastolic volume significantly increased in the control group. Because patients with the greatest damage to their myocardium are the ones who need treatment most, the substudy of these patients was performed. In this sub study the statistically important effect of au tologous transplantation of mononuclear bone marrow cells on myocardial function was not found in patients with moderate-to-severe left ventricular dysfunction. Only an insignificant trend toward the prevention of the left ventric ular dilatation and improvement of global left ventricle ejection fraction was found at 3-month follow-up.

*Study Limitations*

Except the fact that our study is subanalysis, the major limitations of our study are the small number of patients enrolled. However, to this moment it is one of the studies with the highest number of patients with more severe left ven tricular dysfunction ever published. Compared to other studies, the very rigorous myocardial viability assessment was performed before in clusion to this study.

The groups differ slightly in time from on set of infarction to reperfusion. The differences were not statistically significant. The hetero geneity was caused by the inclusion of two pa tients with delayed coronary angioplasty (one patient in the Group H and one patient in the Group C ). In our previous study the biggest effect of cell transplantations was found be tween higher dose and control groups. In this study, the difference in time from infarct on set to reperfusion between Groups H and C was

just 23 minutes. So this difference has not been supposed to affect the results.

Because of ethical consideration, the patients included into the control group did not undergo the identical procedures, as did the bone mar row cell patients, being excluded from the bone marrow aspiration and coronary angiography with the sham cell transplantation. For techni cal reasons, the positron emission tomography (PET) was not performed in all our patients.

In addition to the limited study population, another explanation of our results could be the very severe myocardial damage with almost no surviving myocytes. In these conditions, there is no suitable milieu for catching implanted cells and their differentiation into cardiomyocytes. Also the severe destruction of microcirculation could make the cell homing more difficult com pared to patients with less severe myocardial damage.

**Conclusion**

The important thing is the fact that the selec tion of cells and the whole method of cell ther apy are just at the beginning of the way. Prob ably, it is not realistic to expect some greater changes of left ventricle function in this manner of treatment. It is necessary to look for the best cell type, an optimal way and time of cell deliv ery, and the help of some cytokines. For solving these clinical questions, we must also better un derstand the mechanisms of potential positive effect of the cell therapy.

Taking together, the results of trials show that there is still work to be done to understand a lot of questions related to the cell therapy. Fur ther studies, including larger numbers of pa tients, are needed to resolve all these tasks.

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