

Effect of intramyocardial delivery of autologous bone marrow mononuclear stem cells on the regional myocardial perfusion

NOGA-guided subanalysis of the MYSTAR prospective randomised study

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Summary

The aim of the sub-study of the MYSTAR randomised trial was to analyse the changes in myocardial perfusion in NOGA-defined regions of interest (ROI) with intramyocardial injections of autologous bone marrow mononuclear cells (BM-MNC) using an elaborated transformation algorithm. Patients with recent first acute myocardial infarction (AMI) and left ventricular (LV) ejection fraction (EF) between 30–45% received BM-MNC by intramyocardial followed by intracoronary injection 68 ± 34 days post-AMI (pooled data of MYSTAR). NOGA-guided endocardial mapping and ^{99m}-Sestamibi-SPECT (single photon emission computer tomography) were performed at baseline and at three months follow-up (FUP). ROI was delineated as a best polygon by connecting of injection points of NOGA polar maps. ROIs were projected onto baseline and FUP polar maps of SPECT calculating the perfusion severity of ROI. Infarct size was decreased (from 27.2 ± 10.7% to 24.1 ± 11.5%, $p < 0.001$), and global EF increased (from 38 ± 6.1% to 41.5 ± 8.4%,

$p < 0.001$) three months after BM-MNC delivery. Analysis of ROI resulted in a significant increase in unipolar voltage (index of myocardial viability) (from 7.9 ± 3.0 mV to 9.9 ± 2.7 mV at FUP, $p < 0.001$) and local linear shortening (index of local wall motion disturbances) (from 11.0 ± 3.9% to 12.7 ± 3.4%, $p = 0.01$). NOGA-guided analysis of the intramyocardially treated area revealed a significantly increased tracer uptake both at rest (from 56.7 ± 16.1% to 62.9 ± 14.2%, $p = 0.003$) and at stress (from 59.3 ± 14.2% to 62.3 ± 14.9%, $p = 0.01$). Patients exhibiting ≥5% improvement in perfusion defect severity received a significantly higher number of intramyocardial BM-MNC. In conclusion, combined cardiac BM-MNC delivery induces significant improvement in myocardial viability and perfusion in the intramyocardially injected area.

Keywords

Acute myocardial infarction, stem cells, imaging

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Introduction

Cardiovascular regenerative medicine is on the way to develop cell replacement strategies to re-populate the scar tissue with new contractile cells, building vascularised tissue, in an attempt to restore function of the failing heart (1, 2). Meta-analyses of small, randomised studies using intracoronary adult stem cell injections found a significant improvement in global left ventricular (LV) ejection fraction (EF) between 2.99 and 3.66% (3–5).

In our recently published MYSTAR study (ClinicalTrial.gov Nr. NCT00384982) we have investigated the safety, feasibility and efficacy of combined stem cell application approach using intramyocardial followed by intracoronary delivery of autologous bone marrow mononuclear cells (BM-MNC) (6). The rationale for this strategy is to combine the advantages of both delivery modes: in-

tramyocardial injections allow the strictly targeted delivery of the cells into the borderzone of the infarction and a prolonged retention of the stem cells. On the other hand, intracoronary injections are easy to perform with an infusion catheter and provide the possibility of an unlimited number of injected cells.

We have previously published our observation, that the usual segmental analysis of the left ventricular function or the perfusion defect in the ^{99m}-Tc- MIBI- scintigraphy does not match the local effect of the intramyocardial stem cell injections. To solve the problem, we have developed a NOGA-guided analysis software to determine the change in tracer uptake and myocardial viability in the region of interest (ROI) within the intramyocardially treated area (7).

The aim of the present sub-analysis of the MYSTAR study was to evaluate the local effect of the intramyocardial stem cell therapy

on the targeted area of intramyocardial injections in patients with recent acute myocardial infarction (AMI) and post-infarction cardiac dysfunction. For this sub-analysis we have pooled the data of the MYSTAR population receiving early (32 ± 12 days post-AMI) or late (93 ± 15 days post-AMI) cardiac stem cell therapy, as the main analysis revealed no difference between the groups three months post-BM-MNC therapy as regards the primary or secondary endpoints.

Methods

Patients

The study design, patient inclusion and exclusion criteria and the results on the global left ventricular function and infarct size in the early and late treated groups have been previously published (6, 8). Briefly, 60 patients with recent acute ST-elevation myocardial infarction (STEMI) and re-opened infarct related artery were randomised into two groups. The early intervention group ($n=30$) received the autologous BM-MNC therapy 3–6 weeks (32 ± 12 days) after the STEMI, the late intervention group ($n=30$) received the injections at 3–4 months (93 ± 15 days) post-STEMI.

The main inclusion criteria were the following: age >18 years, global LV EF between 30 to 45%, a new persistent local wall motion disturbance, no significant coronary artery lesion of more than 50% diameter stenosis and a written informed consent. The main exclusion criteria were previous heart surgery or previous MI at the same location, regional wall motion abnormality outside the area involved in the index AMI and severe renal, lung or liver disease. Furthermore patients presenting with severe valvular disease, haemoglobin level below 9 mg%, NYHA class IV or ventricular thrombus were not included either (8).

The primary endpoint of the MYSTAR study was the determination of the changes in resting myocardial perfusion defect size and global LV EF by gated ^{99m}Tc -Sestamibi-SPECT three months after combined BM-SCs therapy. The secondary endpoints included the feasibility, determined by the rate of acute and subacute complications, and safety, expressed as the rate of long-term major adverse cardiac events (death, target vessel revascularisation, and non-fatal myocardial infarction) of the combined stem cell therapy.

Baseline and follow-up investigation included ^{99m}Tc -Sestamibi-SPECT (infarct size, LV EF, end-diastolic volume [EDV], end-systolic volume [ESV]), echocardiography (wall motion score index [WMSI], end-diastolic diameter [EDD], end-systolic diameter [ESD], diameter of the left atrium [LA]) and coronary catheterisation (assessment of coronary lesion, if any, invasive measurement of end-diastolic pressure [EDP]). NOGA endocardial mapping (unipolar voltage [UV] and local linear shortening [LLS] values) was performed at baseline and at the 3-month control after the cell injections (7). Furthermore, clinical parameters (NYHA classification and anginal status [New York Heart Association score] and CCS score [Canadian Cardiovascular Society score])

were recorded at baseline and follow-up. The detailed descriptions of the investigation methods were published previously (6, 8).

BM was harvested from the iliac crest under general anaesthesia, and BM-MNCs were selected. One day later NOGA-guided 3D endocardial map was created after coronary catheterisation. NOGA-guided intramyocardial injections followed by intracoronary administration of the autologous BM-MNCs was performed (7).

^{99m}Tc -Sestamibi-SPECT

All patients underwent a 2-day stress/rest (500–700 MBq ^{99m}Tc -Sestamibi or Tetrofosmin in each study) SPECT protocol at baseline and at the 3-month follow-up (FUP) using dipyridamol infusion over 4–6 min (0.57 mg/kg/min by infusion pump) for the pharmacological stress study. The stress tests at inclusion and at follow-up were performed with identical tracer, exercise loads and cumulative dipyridamol doses.

NOGA mapping and 3D NOGA-guided intramyocardial injections of BM-MNC

The NOGA system components have been previously published (6, 9). Briefly, NOGA technology uses an electromagnetic field created by a triangle beneath the patient to localise the 3D position of the mapping catheter's tip. About 100 measuring points at the endocardium were created to a complete reconstruction of the left ventricle colour-coded according to the myocardial viability expressed in mV or the mechanical function expressed as LLS (9–13).

The intramyocardial injections were targeted into the border zone of the infarction corresponding to a unipolar voltage greater than 6 mV around the scar. About 10 injections of 0.3 ± 0.23 ml diluted stem cell suspension were administered with an injection catheter. A ventricular extrasystole verified intramural position. The remaining 25–26 ml of stem cell suspension were slowly injected into the target vessel having inflated a balloon to avoid immediate washout.

NOGA-guided analysis of the severity of the perfusion defect in the intramyocardial treated area

After automatic inner point and base point filtering of the completed NOGA maps post-injections (at baseline) and post-diagnostic map at FUP, the surface points of the final 3D NOGA map were transposed to the 2D polar map (corresponding internal, middle and outer circles for apex, middle part and base of the left ventricle), using conventional mathematical software converting Cartesian (rectangular) xyz coordinates to polar (r and theta) coordinates, with a theta value of 0 for anterior wall border (► Fig. 1).

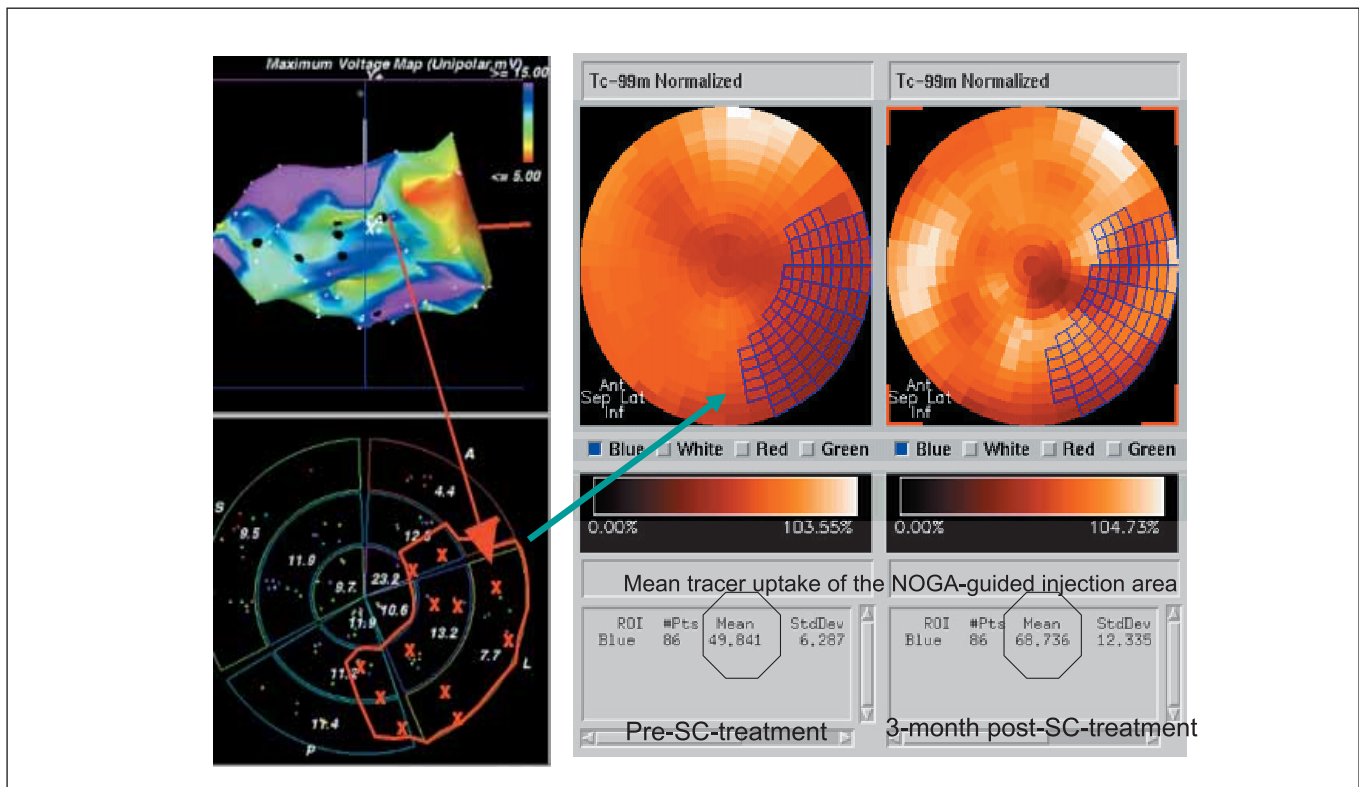


Figure 1: Colour-coded NOGA unipolar voltage image with injection (black) points (left upper panel). Bulls eye view of the NOGA image with delineation of the injected area (region of interest, ROI) (left bottom panel). SPECT image under resting conditions before autologous mononuclear cell (BM-MNC) treatment with the transferred ROI (left scintigraphic polar map) and three months after the cardiac BM-MNC treatment (right scintigraphic polar map), indicating an improvement in mean tracer uptake from 49.8% to 68.7%.

The r -value represents the distance between the point and apex along the long axis of the heart, and theta value represents the angle between the actual point and the centre of the image. The NOGA software constructed both the on-line 3D and the on-line polar maps automatically.

Then the ROI as the best polygon encircling the injection points was drawn using our developed automatic software (7). The mean voltage and LLS values of the ROI were calculated from the numerical values of the mapping points (Fig. 1).

The transaxial files of the baseline and FUP stress and rest SPECT myocardial perfusion imaging (after attenuation and scatter correction) were transferred to an image-analysis workstation (Onyx, SGI, Mountain View, CA, USA), and polar maps were constructed by using Munich-Heart software (14), in a similar way, as for the NOGA polar maps (13). The inter- and intraobserver reproducibility of the Munich heart reconstruction has been published elsewhere (14). Briefly, the inter- and intraobserver variability regression resulted in correlation coefficients >0.908 for calculation of severity, and >0.963 for calculation of extent of perfusion defects, respectively (14).

For transposition of the ROI from the NOGA polar maps to the SPECT polar maps, in order to allow measurements of the size of the injected area and mean normalised tracer uptake of the ROI, an off-line NOGA polar map was reconstructed from the correspond-

ing polar coordinates (r and θ) of each point from the point list by using Deltagraph (DeltaPoint Inc., Monterey, CA, USA). The injected points were then flagged on the reconstructed NOGA polar map, and connected with each other so as to encircle the treated territory as the best concave polygon. On the supposition, that the effect of stem cells is not limited strictly to the injection points, the encircled area was enlarged proportionally by 10%, delineating an extended effective treated area (Fig. 1) (7).

The mean normalised tracer uptake displayed the severity of the perfusion defect under resting and stress conditions and was calculated for the ROI. A tracer uptake above 70% corresponded to normal perfusion, a value between 51% and 70% to a moderate and below 50% to a severe perfusion defect.

Statistics

The results of baseline and follow-up investigations are displayed as mean \pm standard deviation for continuous, and as percentage for categorical variables. A pre-specified sub-analysis was carried out to compare the patients with or without significant ($\geq 5\%$) improvement in mean tracer uptake within the intramyocardially treated area.

Table 1: Baseline characteristics of the patient collective. Values are expressed in mean \pm SD or median (first interquartile range) for continuous parameters and absolute number with percent for categorical variables. AMI: acute myocardial infarction, PCI: percutaneous coronary intervention, GP IIb/IIIa: glycoprotein IIb/IIIa, TIMI: thrombolysis in myocardial infarction, ACE: angiotensin converting enzyme

Characteristics	Patient collective (n=60)
Age (years)	52.7 \pm 10
Male -No (%)	55 (91.7)
Diabetes mellitus-No (%)	17 (28.3)
Hypertension -No (%)	40 (66.7)
Hypercholesterolaemia -No (%)	40 (66.7)
Current smoker -No (%)	31 (51.7)
Coronary artery disease – No (%)	
1-vessel disease -No (%)	49 (81.7)
2-vessel disease -No (%)	8 (13.3)
3-vessel disease -No (%)	3 (5.0)
Infarct-related artery – No (%)	
left anterior descending coronary artery -No (%)	53 (88.3)
left circumflex artery -No (%)	4 (6.7)
right coronary artery -No (%)	3 (5.0)
PCI for additional stenosis of non-infarcted vessel -No (%)	4 (6.7)
Time from symptom onset to first reperfusion therapy (h)	14.4 \pm 5.1
TIMI flow grade before primary PCI	
TIMI flow 0 -No (%)	53 (88.3)
TIMI flow 1 -No (%)	1 (1.7)
TIMI flow 2 -No (%)	3 (5.0)
TIMI flow 3 -No (%)	3 (5.0)
Systemic thrombolysis before PCI -No (%)	3 (5.0)
GP IIb/IIIa antagonist during primary PCI -No (%)	13 (21.7)
Implantation of drug-eluting stent in infarct-related artery -No (%)	55 (91.7)
TIMI flow 3 grade after primary PCI -No (%)	59 (98.3)
Maximal creatine kinase (U/l)	3676 \pm 719
Haematocrite before harvesting of bone marrow (%)	42.0 \pm 4.3
Haemoglobin before harvesting of bone marrow (g/dl)	12.5 \pm 3.8

Characteristics	Patient collective (n=60)
Before intramyocardial stem cell therapy	
Creatine kinase (U/l)	141 \pm 29
Troponin T (ng/ml)	0.01 (0; 0.02)
After intramyocardial stem cell therapy	
Maximal creatine kinase (U/l)	163 \pm 31
Maximal troponin T (ng/ml)	0.01 (0; 0.02)
Before control NOGA mapping	
Creatine kinase (U/l)	99 \pm 51
Troponin T (ng/ml)	0.0 (0; 0.02)
After control NOGA mapping	
Maximal creatine kinase (U/l)	136 \pm 31
Maximale troponin T (ng/ml)	0.0 (0; 0.01)
Medication at primary discharge -No (%)	
Aspirin -No (%)	60 (100)
Clopidogrel -No (%)	60 (100)
Warfarin -No (%)	1 (1.7)
Beta-blockers -No (%)	28 (91.7)
ACE inhibitors or angiotensin-receptor blockers -No (%)	58 (96.7)
Statins -No (%)	58 (96.7)
Diuretics -No (%)	23 (38.3)
Medication at control	
Aspirin -No (%)	60 (100)
Clopidogrel -No (%)	60 (100)
Warfarin -No (%)	1 (1.7)
Beta-blockers -No (%)	54 (90)
ACE inhibitors or angiotensin-receptor blockers -No (%)	55 (91.7)
Statins -No (%)	57 (95)
Diuretics -No (%)	14 (23.3)

For comparison of the continuous variables, a Student's t-test, for categorical variables chi-square tests were calculated. A p value <0.05 was considered statistically significant.

Results

Patients

The mean age of the patients was 52.7 \pm 10.6 y, 91.7% of the patients were male. The combined (intramyocardial followed by intracoronary) delivery of the BM-MNC was performed mean 68 \pm

34 days post-AMI. ► Table 1 lists the baseline characteristics for the entire patient population.

Clinical, echocardiographic and ventriculographic parameters

CCS and NYHA had improved significantly at three months after BM-MNC therapy. Enlargement of the LA was diminished significantly three months post-BM-MNC injections, while a trend towards decrease in LV EDD was measured. Mild improvement in WMSI, and LV EDP was observed (► Table 2).

Table 2: Effect of the stem cell therapy on clinical, echocardiographic and angiographic parameters. Pooled analysis (n=60) of the MYSTAR study including patients receiving combined (intramyocardial followed by intracoronary injections) of autologous unselected bone marrow mononuclear cells (BM-MNC) post-myocardial infarction. BM-MNC: bone marrow mononuclear cells, NYHA: Score of the New York Heart Association, CCS: Score of Canadian Cardiovascular Society, LA: diameter of left atrium, EDD: end-diastolic diameter of the left ventricle, WMSI: wall motion score index, EDP: end-diastolic pressure of the left ventricle.

	Before BM-MNC therapy	Three months after BM-MNC therapy	P-value
Clinical data			
NYHA	2.0 ± 0.9	1.4 ± 0.6	<0.001
CCS	1.8 ± 0.7	1.2 ± 0.5	<0.001
Transthoracic echocardiography			
LA [mm]	53.6 ± 9.0	48.3 ± 7.8	<0.001
EDD [mm]	54.2 ± 7.0	51.9 ± 7.3	0.070
WMSI	1.8 ± 0.5	1.7 ± 0.4	0.200
Ventriculography			
EDP [mmHg]	23.4 ± 7.7	20.5 ± 8.8	0.186

Table 3: Results of the gated ^{99m}Tc-MIBI SPECT images. Pooled analysis study (n=60) of the scintigraphic data of the MYSTAR study patients receiving combined (intramyocardial followed by intracoronary injections) of autologous unselected BM-MNCs post-myocardial infarction. BM-MNC: bone marrow mononuclear cells, EF: ejection fraction, ESV end-systolic volume, EDV: end-diastolic volume.

	Before BM-MNC therapy	Three months after BM-MNC therapy	P-value
Infarct size [%]	27.2 ± 10.7	24.1 ± 11.5	<0.0001
EF [%]	38.0 ± 6.1	41.5 ± 8.4	<0.0001
ESV [ml]	166.9 ± 78.2	137.6 ± 36.1	0.002
EDV [ml]	206.9 ± 69.2	207.4 ± 75.4	0.764

^{99m}-Sestamibi-SPECT

The global evaluation of the ^{99m}Tc-MIBI scintigraphies revealed a significant reduction in the infarct size calculated as infarcted sub-segments in the bulls-eyes view as a percentage of the whole myocardium. Global LV EF increased, while ESV decreased significantly. LV EDV did not change after stem cell injections (► Table 3).

NOGA-guided analysis of the intramyocardially injected area

A significant increase in the electric as well as in the mechanic properties in the intramyocardially treated area of the left ventricle

was documented three months after the combined delivery of the BM-MNCs (► Fig. 2). Both the unipolar voltage (index of myocardial viability) (from 7.9 ± 3.0 mV to 9.9 ± 2.7 mV at FUP, p<0.001) and LLS (index of local wall disturbances) (from 11.0 ± 3.9 to 12.7 ± 3.4, p=0.01) improved significantly. NOGA-guided analysis of the intramyocardially treated area revealed a significantly increased tracer uptake both at rest (from 56.7 ± 16.1% to 62.9 ± 14.2%, p=0.003) and at stress (from 59.3 ± 14.2% to 62.3 ± 14.88%, p=0.01) (► Fig. 3).

Sub-analysis of patients with significant improvement in tracer uptake after cardiac BM-MNC therapy

In the sub-analysis we have investigated the clinical, echocardiographic, angiographic and the NOGA-guided SPECT parameters of the patients gaining ≥5% improvement (23 patients, 38%) in mean tracer uptake in the NOGA-guided intramyocardially injected area (Table 3). We found no difference between the patients with/without ≥5% improvements as regards the baseline clinical (age, time between AMI and cardiac BM-MNC therapy, CCS and NYHA classification), echocardiographic (LA, EDD, WMSI), angiographic (location of the infarct-related artery, EDP), or SPECT-derived parameter (EDV, ESV, EF and infarct size). However, patients displaying significant improvement in infarct severity received a significantly higher number of intramyocardially injected BM-MNC. Interestingly, the number of the intramyocardially injected CD34+ cells, or the amount of cells injected intracoronary did not influence the severity of the infarction (Table 3). Patients showing significant improvement in tracer uptake had initially more profound tracer deficit with similar infarct size (Table 3).

Discussion

This sub-analysis of the pooled data of MYSTAR combined delivery of BM-MNC therapy revealed a significant improvement in electric and mechanical function as well as in the myocardial perfusion of the intramyocardially injected area. In contrast to the standard techniques our software with the transformation of the injection area (ROI) to the scintigraphic images allows to analyse the local effect of the intramyocardial stem cell therapy. The used algorithm permits a detailed quantitative analysis of the severity of a specifically treated area, and thus it furnishes more information than the global or the usual segmental artery-driven quantitative, or the visual semi-quantitative evaluation of SPECT images; thus it can be a useful tool for analysis of intramyocardial cell-, gene- or cell-based gene therapy in general. In spite of the significant increase in tracer uptake both at stress and rest in the entire patient collective of MYSTAR study, the perfusion abnormality did not reach the normal values, and only 38% of the patients exhibited an individual significant improvement in rest-induced defect severity on myocardial scintigraphy. These data are in accordance with the

Figure 2: Representative NOGA-images of a 45-year-old male patient, post-anterior wall myocardial infarction. NOGA-guided intramyocardial injections in the border zone of the infarction (black points). Three months after autologous BM-MNC therapy, the size of the ventricle is decreased, suggesting a reverse remodeling.

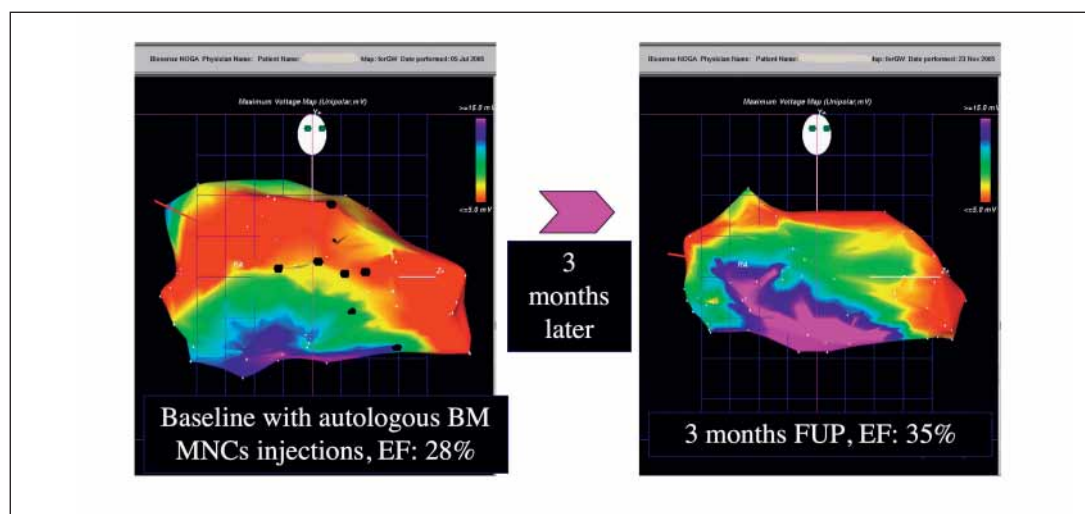


Table 4: Effect of the intramyocardial bone marrow mononuclear cells (BM-MNC) therapy on the clinical, echocardiographic, angiographic and NOGA-guided resting SPECT parameters. Pooled analysis (n=60) of the MYSTAR study including patients receiving combined (intramyocardial followed by intracoronary injections) of BM-MNC post-myocardial infarction.

	Improvement in tracer uptake $\geq 5\%$ YES (n=23)	Improvement in tracer uptake $\geq 5\%$ NO (n=37)	P-value
Resting tracer uptake [%]	49.2 \pm 14.7	62.0 \pm 15.1	0.004
Baseline Voltage value [mV]	7.1 \pm 2.3	8.4 \pm 3.3	0.134
Baseline LLS value [%]	11.2 \pm 3.2	10.9 \pm 4.4	0.798
Days after AMI	56.0 \pm 32.7	66.4 \pm 33.7	0.243
Age	52.9 \pm 9.2	52.5 \pm 11.4	0.919
NYHA at baseline	1.9 \pm 1.0	2.0 \pm 0.9	0.779
Number of totally intramyocardially injected cells [10^9]	0.22 \pm 0.1	0.18 \pm 0.1	0.008
Number of intramyocardially injected CD34+ cells [10^6]	1.8 \pm 2.2	1.9 \pm 1.9	0.797
Number of totally intracoronarily injected cells [10^9]	1.1 \pm 1.2	1.3 \pm 1.2	0.635
Number of intracoronarily injected CD34+ cells [10^6]	13.24 \pm 22.15	20.73 \pm 26.62	0.273
Baseline WMSI	1.9 \pm 0.6	1.8 \pm 0.4	0.238
Baseline EDP [mmHG]	23.1 \pm 8.4	23.5 \pm 7.5	0.879
Baseline EDD [mm]	53.1 \pm 5.9	55.0 \pm 7.6	0.370
Baseline diameter of left atrium [mm]	54.3 \pm 8.3	53.1 \pm 9.5	0.678
Baseline CCS	1.7 \pm 0.8	2.0 \pm 0.7	0.316
Time from AMI to reperfusion [h]	13.1 \pm 3.9	15.3 \pm 5.6	0.079
Baseline EF in scintigraphy [%]	37.7 \pm 5.8	38.2 \pm 6.3	0.749
Baseline EDV in scintigraphy [ml]	208.0 \pm 71.3	206.3 \pm 69.1	0.935
Baseline ESV in scintigraphy [ml]	156.3 \pm 72.7	172.5 \pm 81.5	0.495
Baseline rest extend of AMI in scintigraphy [%]	25.6 \pm 12.1	28.2 \pm 9.8	0.363
Risk factor diabetes mellitus (n=18)	7	11	0.588
Risk factor hypertension (n=41)	16	25	0.552
Risk factor dyslipidemia (n=41)	17	24	0.330
Risk factor smoking (n=19)	14	15	0.257
Infarct-related artery LAD (n=52)	18	34	0.245

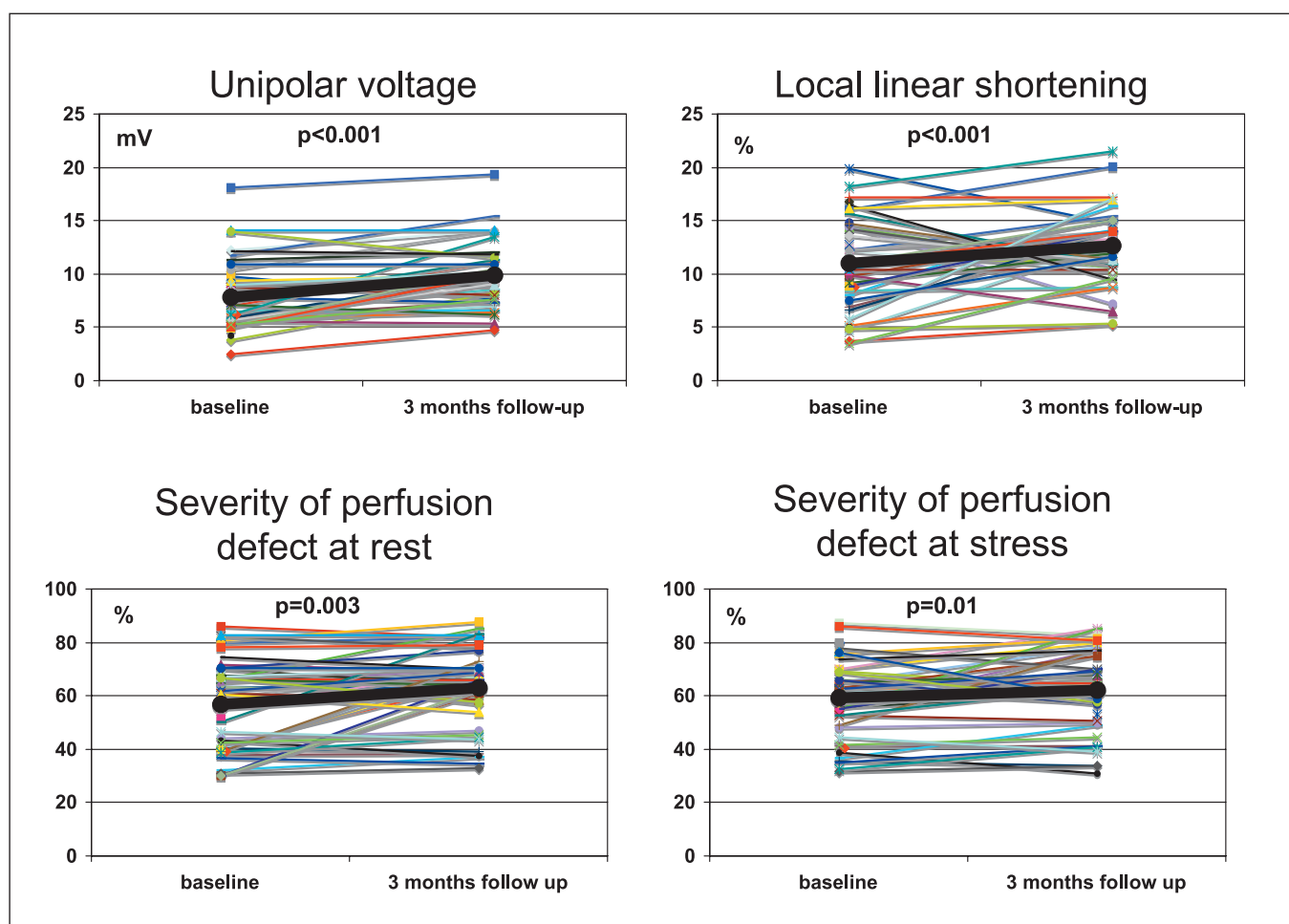


Figure 3: Results. NOGA-guided analysis of the myocardial viability (expressed as unipolar voltage), local wall motion abnormality (expressed as local linear shortening), tracer uptake at rest and at stress in the intramyocardially injected area.

results of the previously published MYSTAR study as regards the significant improvement in global EF and infarct size in approximately 33–43% of patients, regardless whether the patients received the cardiac BM-MNC 3–6 weeks or 3–4 months post-AMI.

Compared to other clinical studies using autologous stem cell therapy after AMI, the effect of this novel approach on the global LV function lies in the same range. While some groups could not detect a positive influence of the injected cells on myocardial regeneration (15, 16), most others reported an increase in LV EF between 2 and 6% (17–21). In contrast to our study, all of these centres used the intracoronary delivery mode. All of the mentioned studies performed the stem cell therapy within 10 days after the infarction, even if the highly inflammatory milieu in the acute setting is not favourable for cells surviving and homing.

The intramyocardial delivery of the stem cells is a time- and cost-consuming procedure, using the 3D NOGA guidance. Up to now, there are only limited numbers of publications reporting the effect of percutaneous intramyocardial delivery of stem cells on cardiac perfusion and function. Krause et al. have recently published a significant increase in LV EF from $40.8 \pm 6.9\%$ to $47.1 \pm$

10.6% after NOGA-guided injections of autologous BM-MNC; albeit without standardised assessment of LV EF with MRI or gated scintigraphy (22). Losordo et al. reported a favourable trend as regards the efficacy parameters including angina frequency, nitroglycerine usage, exercise time, and Canadian Cardiovascular Society in percutaneous intramyocardial CD34+ cell-treated patients versus control subjects given placebo (23). The surgical intramyocardial delivery of stem cells without bypass surgery led to similar results, gaining an increase in global LV EF between 4.8% to 9% (24, 25) in patients with ischaemic cardiomyopathy. To our knowledge, we are the only centre combining both delivery modes in patients after AMI. The specific analysis of the intramyocardially treated area revealed a mean increase in tracer uptake of 6.2%; with 38% of patients exhibiting an individual significant improvement ($\geq 5\%$) in perfusion severity in the intramyocardial area; without taking into consideration the possible effect of the additional intracoronary delivery. As the magnitude of the improvement is in concordance with the global changes, we suppose that the majority of the benefit of the cardiac stem cell therapy is related to the intramyocardial delivery; although the additional effect of the intracor-

onary stem cell delivery should also be taken into account. The myocardial perfusion imaging with SPECT is the only useful tool for the evaluation of the lesion volume (extent) and reduction in the blood perfusion (severity) of a mal-perfused myocardial area (26, 27). Analysis of these two parameters allows to separate the transmural and subendocardial lesions with the same extent, as the severity of the perfusion defect differs. Thus, the NOGA-guided scintigraphic analysis is a useful tool for exact evaluation of the intramyocardial regeneration therapy.

In conclusion, the sub-analysis of the pooled data of MYSTAR combined delivery of BM-MNC therapy revealed a significant improvement in electric and mechanical function as well as in the myocardial perfusion of the intramyocardially injected area. The specific analysis of the intramyocardially treated area revealed a mean increase in tracer uptake of 6.2%; with 38% of patients exhibiting an individual significant improvement ($\geq 5\%$) in perfusion severity. The NOGA-guided scintigraphic analysis allows an exact evaluation of the local effect of the intramyocardial stem cell therapy, thus it can be a useful tool for analysis of intramyocardial cell-, gene- or cell-based gene therapy in general.

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