Improving risk stratification after acute myocardial infarction : focus on emerging applications of echocardiography

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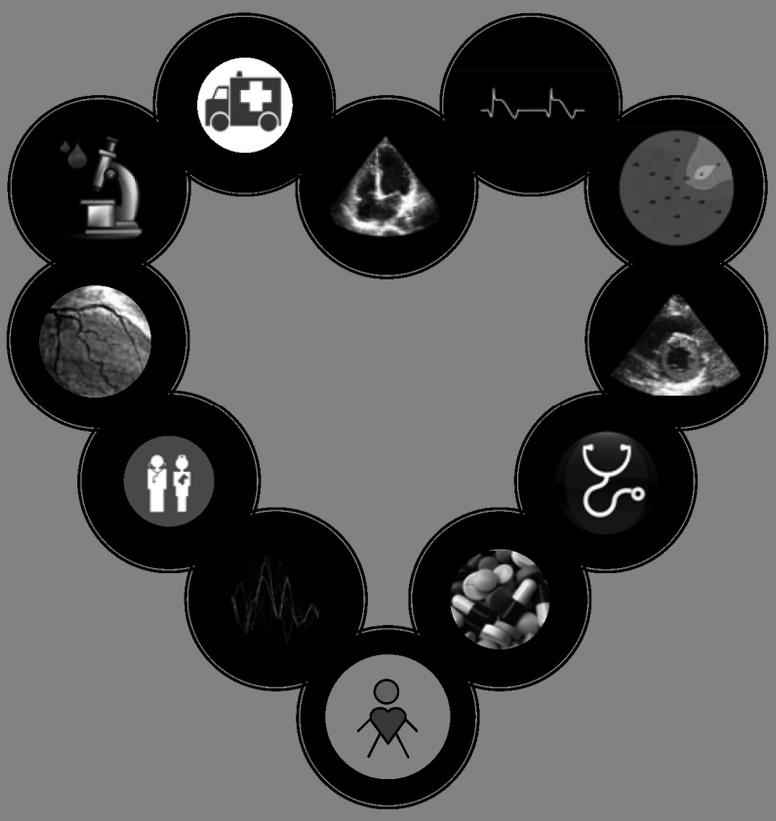

Chapter 17

Intramyocardial Bone Marrow-derived Mononuclear Cell Injection for Chronic Myocardial Ischemia: the Effect on Diastolic Function

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Abstract

Objectives

The present substudy of a recently published randomized trial aimed to investigate the effect of intramyocardial bone marrow cell injection on diastolic function in patients with chronic myocardial ischemia.

Methods and results

In a total of 50 patients, diastolic function was evaluated before and 3 months after bone marrow cell injection using standard echocardiography and strain analysis. In addition, magnetic resonance imaging-derived transmitral flow measurements were obtained in a subset of 36 patients. Left ventricular ejection fraction increased from 50 ± 5% to 54 ± 7% in the bone marrow cell group, which was a significant improvement as compared to the placebo group (52 ± 5% vs. 51 ± 7%, p = 0.001). Filling pressure estimate E/E' ratio improved from 14 ± 5 at baseline to 12 ± 4 at 3 months in the bone marrow cell group, whereas no improvement was observed in the placebo group (13 ± 4 vs. 13 ± 5). The improvement in E/E' ratio was significantly larger in the bone marrow cell group (p = 0.008). Furthermore, the E/A peak flow ratio as assessed by MRI showed a significant increase in the bone marrow cell group as compared to the placebo group (+0.16 ± 0.25 vs. -0.04 ± 0.21, p = 0.01), which was mainly related to an increase in the early (E) peak flow rate in the bone marrow cell group (from 407 ± 96 mL/sec to 468 ± 110 mL/sec, p = 0.009 as compared to placebo group).

Conclusions

The current study demonstrates that intramyocardial bone marrow cell injection is associated with a beneficial effect on myocardial relaxation and filling pressures in patients with chronic myocardial ischemia.

Introduction

Bone marrow cell therapy has emerged as a potential therapeutic option for patients with chronic ischemic heart disease. Nonrandomized clinical studies demonstrated that intramyocardial injection of bone marrow-derived mononuclear cells is safe and feasible in patients with chronic myocardial ischemia.[1] Moreover, our recently published randomized, double-blinded, placebo-controlled trial[2] revealed that intramyocardial bone marrow cell injection is associated with improvements in myocardial perfusion and left ventricular (LV) systolic function in patients with chronic myocardial ischemia. These improvements were paralleled by a reduction in anginal complaints and an increased quality of life. Experimental and clinical data have suggested that bone marrow cell injection may also have a beneficial effect on diastolic function.[3-6] Diastolic dysfunction is often present in patients with coronary artery disease and is an important prognostic factor.[7] Therefore, more detailed determination of the effects on diastolic function is needed to further explore the functional benefit of bone marrow cell injection. In addition, such information could provide mechanistic insights and may have implications for future studies. In a non-randomized clinical study in patients with chronic myocardial ischemia, improvement in parameters of diastolic function was suggested after intramyocardial bone marrow cell injection.[8] On the basis of these results, the current substudy was conducted to investigate the effect of intramyocardial bone marrow cell injection on LV diastolic function in a randomized, double-blinded, placebo-controlled setting. Diastolic function was evaluated by echocardiographic measurement of parameters of filling pressure, myocardial relaxation and ventricular compliance with the use of tissue Doppler imaging (TDI) and speckle tracking strain analysis. In addition, changes in the transmitral flow pattern were evaluated using magnetic resonance imaging (MRI).

Methods

Patient selection and study protocol

The detailed study protocol of this randomized, double-blinded, placebo-controlled trial has been described previously.[2] In brief, patients with chronic ischemic heart disease were eligible for inclusion if they had refractory angina (Canadian Cardiovascular Society (CCS) class III or IV despite optimal medical therapy) and stress-inducible ischemia on

technetium-99m tetrofosmin single photon emission computed tomography (SPECT). In all patients, bone marrow was aspirated from the iliac crest. Mononuclear cells were isolated by Ficoll density gradient centrifugation. Following cell isolation, patients were randomized in a 1:1 ratio to either intramyocardial injection of $100x10^6$ autologous bone marrow-derived mononuclear cells or placebo solution (NaCl 0.9% with 0.5% human albumin). Intramyocardial injections were targeted at myocardial regions with stress-inducible ischemia (on SPECT) and were performed using NOGA system (BDS, Cordis, California, USA) during cardiac catheterization.[2] The protocol was approved by the institutional ethics committee and complied with the declaration of Helsinki. The study was registered at the Dutch trial registry (www.trialregister.nl, no. NTR400/ISRCTN58194927). Our primary end-point was defined as the improvement in filling pressure estimate E/E' ratio. Furthermore, an extensive evaluation of diastolic function was performed using TDI and speckle tracking strain analysis on echocardiography. Moreover, changes in the transmitral flow pattern of the left ventricle were assessed using magnetic resonance imaging (MRI).

Echocardiography

Images were obtained using a commercially available system (Vivid Seven, General Electric-Vingmed, Milwaukee, Wisconsin, USA). Color Doppler frame rates ranged between 115-160 frames per second (mean 148 frames per second), depending on the sector width of the range of interest, and pulse repetition frequencies were between 500 Hz and 1 kHz, resulting in aliasing velocities between 16 and 32 cm/s. The anteroposterior diameter of the LA was measured at end-systole on the M-mode image obtained from the parasternal long-axis view. LA volume was calculated using the biplane method.[9] LV volumes and ejection fraction were derived using the biplane Simpson's rule.[10] Mitral inflow was analyzed using peak early filling velocity (E), deceleration time of early filling velocity, and peak atrial filling velocity (A). Pulmonary venous flow was recorded placing a sampling volume 1cm in the right upper pulmonary vein, to assess peak systolic velocity (S) and peak diastolic velocity (D).

Tissue doppler imaging

TDI parameters were measured by two independent observers from color-coded images of 3 consecutive heartbeats by offline analysis. Early diastolic velocity (E') was measured at 4 LV basal segments by placing the sample volume in the LV basal portion of the septum, the lateral wall (using the 4-chamber view), the anterior wall, and the posterior wall (using the 2-chamber view). The described E' value is the average value of these 4 measurements. Late diastolic velocity (A') was measured in the LV basal portion of the septum. The respective intra- and inter-observer variabilities were as follows: 0.1±0.1 cm/sec and 0.4±0.1 cm/sec for septal E'; 0.1 ± 0.2 cm/sec and 0.4 ± 0.1 cm/sec for A'; and 0.01 ± 0.02 and 0.02 ± 0.02 for the E'/A' ratio.[8]

Speckle-tracking strain and strain rate analysis

Two-dimensional speckle-tracking imaging allows angle-independent analysis of myocardial deformation.[11] Myocardial strain is a measure for the change in myocardial length and is presented as a percentage of the initial length.[12] Strain and strain rate quantification was performed as previously described,[11,13] by using a commercially available software (EchoPAC version 108.1.5, General Electric-Vingmed). Longitudinal strain and strain rate were assessed from the apical long-axis view and the 2- and 4-chamber views. The global strain rate value was defined as the average of the peak systolic strain rate values of 18 segments. For strain rate measurements, the software divides the apical segment in 2 segments, creating the 18th segment. Since the injected and non-injected segments had been pre-defined using the generally accepted 17-segment model,[14] the 2 apical segments were merged to 1 segment by averaging the strain rate values for comparison of injected and non-injected segments. Thus, 1 apical segment was created which was geometrically identical with the apical segment in the 17-segment model used for strain analysis. For assessment of diastolic strain rate indices, peak global strain rate during the isovolumic relaxation (GSR_{IVR}) period and during early diastole (GSR_E) were measured and averaged from the 3 apical views.[15] By calculating the ratio of peak transmitral E wave to GSR_{IVR} (E/GSR_{IVR}), a novel parameter for estimation of LV filling pressure was obtained.[15]

Magnetic resonance imaging

A 1.5-T Gyroscan ACS-NT MRI scanner (Philips Medical Systems, Best, the Netherlands) equipped with Powertrack 6000 gradients and 5-element cardiac synergy coil was used. With the use of a balanced, fast-field echo sequence with parallel imaging (SENSE, acceleration factor 2), the heart was imaged from apex to base, with 10 to 12 imaging levels (dependent on the heart size) in the short-axis view. Typical parameters were a field of view of 400 x 400 mm2, matrix of 256 x 256 pixels, slice thickness of 10 mm, no slice gap, flip angle of 50°, time to echo of 1.82 ms, and time to repeat of 3.65 ms. The temporal resolution was 25 to 39 ms. Phase contrast flow velocity measurements across the mitral valve orifice were obtained using a fast-field echo sequence with retrospective gating. Velocity maps were acquired across the mitral orifice using a flip angle of 20° and an echo time of 10 to 12 ms. The image section had a thickness of 8 mm, a field of view of 350 x 350 mm2, and consisted of two measurements of a 128 x 128 acquisition matrix which was interpolated to a display matrix of 256 x 256 pixels. Between 30 and 45 time frames (depending on the actual heart rate) were evenly distributed over the cardiac cycle, resulting in a temporal resolution of 25 to 30 ms. Total acquisition time was about 3 min. The maximum phase shift of 180° was set to occur at a velocity of 100 cm/s. For a comprehensive diastolic function assessment, volumetric flow across the mitral valve was calculated by manually tracing the borders of the mitral valve in all time frames of the velocity map series, using the FLOW analytical software package (Medis, Leiden, the Netherlands). Automatic analysis of flow curves was performed following a manual indication of the start of early filling, peak early filling, peak atrial contribution to filling, and the end of filling as previously described[16]. The intra- and interobserver variabilities were 1±4 mL/sec and 2±7 mL/sec for the early (E) peak flow rate; 2±4 mL/sec and 3±7 mL/sec for the late (A) peak flow rate; and 0.01±0.02 and 0.02±0.03 for the E/A ratio, respectively.[8]

Statistical analysis

Data are reported as mean±SD. Comparison of continuous data was performed using the Student t-test and categorical variables were compared using the chi-square test or Fisher's exact test. Repeated measures analysis of variance was used to study the relation between

the allocated treatment and (changes in) continuous data at baseline and 3 months follow-up. All analyses were performed in line with the intention-to-treat principle. For all paired tests, complete case analysis was performed. A P-value <0.05 was considered significant. Statistical analyses were performed with SPSS software (version 16.0, SPSS) and SAS statistical software (SAS Institute, Cary, North Carolina).

We applied linear regression analyses for the evaluation of (changes in) segmental strain and strain rate in relation to treatment allocation. We used the segment (and not the patient) as unit of our analyses, and each patient contributed with 17 segments. We realized that the observations within a patient might not be entirely independent, and we used the method of generalized estimating equations to account for clustered data. Two linear regression models were developed, with the absolute changes in myocardial strain and strain rate as dependent variable, and treatment group and a variable that indicated if a segment was injected or not as determinants. We evaluated if the relation between injected vs. noninjected segments and the absolute changes in myocardial strain and strain rate was modified by treatment allocation by including an interaction term in the model. Changes in strain and strain rate between injected segments and noninjected segments were further evaluated in both treatment groups separately.

Results

A total of 50 patients were randomly assigned to receive bone marrow cell injection (n = 25) or placebo injection (n = 25). The baseline characteristics of these patients are shown in Table 1. The type and dose of medications remained unchanged during the entire study period. Systolic and diastolic blood pressures did not change during follow-up in both groups (bone marrow cell group 122 ± 22 mmHg systolic (p = 0.63) and 79 ± 10 mmHg diastolic (p = 0.59), placebo group 115 ± 17 mmHg (p = 0.15) and 78 ± 10 mmHg (p = 0.26), respectively).

Procedural data and clinical outcome data of the cohort have been previously reported[2]. One patient died at 2.5 months follow-up because of myocardial ischemia leading to acute heart failure. Therefore, paired echocardiography at baseline and 3 months follow-up was available in 24 patients in the bone marrow cell group and 25 patients in the placebo group. Paired data of A, E/A ratio, A', and E'/A' ratio were available in 22 patients in the cell

group and 24 in the placebo group, due to atrial fibrillation in 2 bone marrow-cell treated patients and 1 placebo-treated patient.
MRI at baseline and 3 months follow-up was available in 22 patients in the cell group and in 18 patients in the placebo group. Atrial fibrillation or insufficient flow measurements quality prevented reliable assessment of diastolic function in 3 patients in the cell group and 1 patient in the placebo group, as illustrated in the flow chart (Figure 1).

Table 1. Baseline characteristics of the study population

	Bone marrow cell group (N = 25)		***Placebo group (N = 25)***	
Age, years	64 ± 8		62 ± 9	
Gender (Male)	23	(92%)	20	(80%)
Diabetes	13	(52%)	8	(32%)
Smoking	10	(40%)	12	(48%)
Dyslipidemia	12	(48%)	15	(60%)
Hypertension	12	(48%)	11	(44%)
Coronary artery disease in family	16	(64%)	13	(52%)
Systolic blood pressure (mmHg)	122 ± 20		121 ± 22	
Diastolic blood pressure (mmHg)	78 ± 7		77 ± 10	
Beta-blockers	24	(96%)	24	(96%)
Statins	25	(100%)	25	(100%)
Calcium channel blockers	18	(72%)	18	(72%)
Nitrates	21	(84%)	21	(84%)
ACE inhibitors	19	(76%)	14	(56%)
Prior Myocardial infarction	14	(56%)	18	(72%)
Prior CABG	24	(96%)	19	(76%)
Prior PCI	16	(64%)	13	(52%)

Global and regional systolic function

After 3 months follow-up, LV ejection fraction increased from 50 ± 5% to 54 ± 7% in the bone marrow cell group, which was a significant improvement as compared to the placebo group ($p = 0.001$). The increase in LV ejection fraction was mainly related to a decrease in LV end-systolic volume, since no changes were observed in LV end-diastolic volume

(Table 2). Table 3 summarizes the results of the evaluation of regional myocardial function. The observed improvements in global strain and global strain rate were significantly larger in the bone marrow cell group as compared to the placebo group (both p = 0.04). Using the linear regression model, no significant interaction was observed between treatment allocation and changes in segmental myocardial strain and strain rate, since interaction terms were not significant (p = 0.07 and p = 0.23, respectively). Nonetheless, within-group analysis revealed that in bone marrow cell-treated patients, improvement in myocardial strain was significantly larger in injected segments as compared to noninjected segments (p = 0.008, Table 3). Similarly, the increase in myocardial strain rate was significantly greater in injected segments as compared to noninjected segments (p = 0.01). In placebo-treated patients, improvements in myocardial strain and strain rate were not significantly larger in the injected segments as compared to the noninjected segments (p = 0.81 and p = 0.25 respectively).

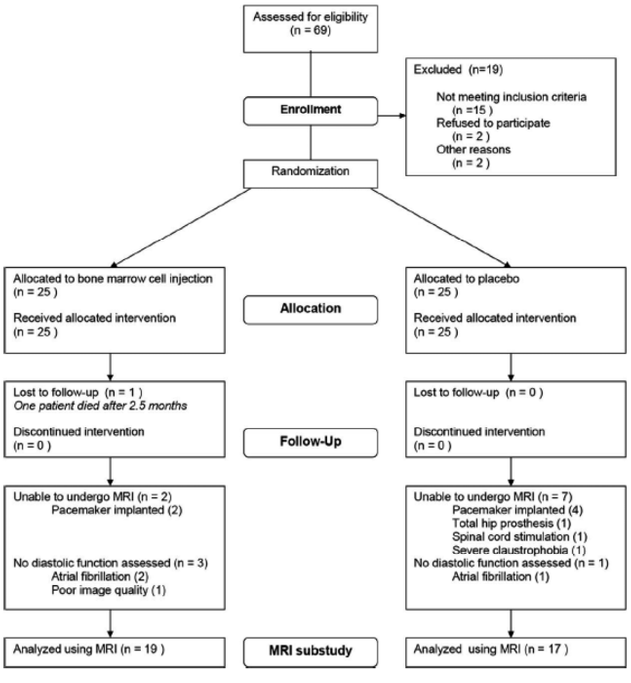

Figure 1: Flow chart of participants through the study.

Parameters of diastolic function with echo

At 3 months follow-up, filling pressure estimate E/E' ratio improved significantly in the bone marrow cell group as compared with the placebo group ($p = 0.008$), as shown in Figure 2. No changes in left atrial size were detected in both treatment groups (Table 2). A trend toward improvement was observed in transmitral E/A ratio ($p = 0.06$). As shown in table 2, this was mainly related to an increase in transmitral E-wave in bone marrow cell-treated patients. E wave deceleration time and pulmonary venous flow patterns did not change in both groups. Early diastolic velocity (E') improved after bone marrow cell injection ($p = 0.0001$), whereas A' remained unchanged ($p = 0.42$), resulting in a significant increase in E'/A' ratio in the bone marrow cell group as compared to the placebo group ($p = 0.0001$, Table 2).

Paired measurements of GSR_{IVR} and, consequently, filling pressure estimate E/GSR_{IVR} were available in 18 patients in the bone marrow cell group and in 22 patients in the placebo group. GSR_E was available in all patients. Compared with placebo treated-patients, GSR_{IVR} increased significantly in bone marrow cell-treated patients at 3 months follow-up ($p = 0.0005$), resulting in a trend toward improvement in filling pressure estimate E/GSR_{IVR} ($p = 0.06$). No significant changes were observed in GSR_E (Table 2).

Table 2. Echocardiographic measurements

	BMC group		***Placebo group***		***Change***		***P***
	Baseline	***3 months***	***Baseline***	***3 months***	***BMC***	***Placebo***	
LVEDV(ml)	102±36	101±38	101±31	100±30	-1±15	-1±27	0.94
LVESV (ml)	51±23	47±26	49±17	51±22	-4±8	2±16	0.10
LVEF(%)	50±5	54±7	52±5	51±7	4±4	-1±7	0.001
LA (ml)	46±13	49±12	48±16	46±14	3±10	-2±11	0.16
E, (cm/s)	69±16	73±16	66±16	66±19	4±10	0±13	0.27
A, (cm/s)	72±18	70±18	70±17	71±20	-2±11	1±12	0.88
E/A ratio	1.0±0.3	1.1±0.2	1.0±0.3	1.0±0.3	0.1±0.2	-0.0±0.2	0.06
DT	200±59	209±63	211±59	213±72	9±61	2±69	0.74
S/D ratio	1.3±0.3	1.3±0.3	1.2±0.3	1.3±0.2	0±0.3	0.1±0.3	0.43
E', (cm/s)	5.5±1.4	6.4±1.5	5.6±1.5	5.2±1.3	0.9±1.1	-0.4±1.0	0.0001
A', (cm/s)	7.3±1.5	7.0±1.4	6.6±1.2	6.6±1.3	-0.3±1.3	-0.0±1.1	0.42
E/E' ratio	14±5	12±4	13±4	13±5	-2±3	0±3	0.008
E'/A' ratio	0.8±0.2	0.9±0.2	0.9±0.3	0.8±0.3	0.1±0.2	-0.0±0.2	0.0001
GSR_{IVR} (s^{-1})	0.2±0.1	0.4±0.2	0.3±0.2	0.2±0.1	0.2±0.1	-0.0±0.2	0.0005
GSR_{E} (s^{-1})	0.9±0.3	1.0±0.3	0.9±0.3	0.9±0.3	0.0±0.2	-0.0±0.2	0.41
E/GSR_{IVR}	376±209	259±178	355±317	378±274	-109±144	23±263	0.06

LV: left ventricular; E: early diastolic filling velocity, A: late diastolic filling velocity, S: pulmonary vein peak systolic velocity, D: pulmonary vein peak diastolic velocity: IVR: isovolumetric relaxation.

Table 3. Global and regional myocardial strain evaluation

	Bone marrow cell group			*Placebo group*		
	Global	***Injected***	***Noninjected***	***Global***	***Injected***	***Nonjected***
Strain						
Baseline	-15.5±4.1	-12.5±6.1	-15.4±6.3	-16.2±3.0	-14.1±6.2	-16.3±6.0
3 months	-16.9±4.1	-15.7±7.0	-16.4±6.5	-16.1±3.5	-14.3±6.2	-16.3±5.9
Change	-1.4±2.0	-3.2±6.1	-1.0±5.5	0.1±2.6	0.2±4.0	0.0±2.5
		p = 0.008			p = 0.81	
Strain						
Baseline	-0.95±0.22	-0.83±0.43	-0.97±0.36	-0.99±0.18	-0.89±0.33	-1.01±0.38
3 months	-1.09±0.25	-1.08±0.42	-1.09±0.44	-1.02±0.23	-0.95±0.42	-1.03±0.39
Change	-0.14±0.18	-0.25±0.49	-0.12±0.42	-0.03±0.20	-0.06±0.32	-0.02±0.19
		p = 0.01			p = 0.25	

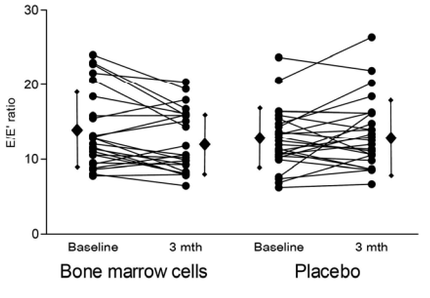

Figure 2.

In the bone marrow cell group, filling pressure estimate E/E' ratio improved from 14±5 to 12±4. In the placebo group, no improvement was observed (13±4 vs. 13±5). The improvement in the bone marrow cell group was significantly larger compared to the placebo group (p = 0.008).

Parameters of diastolic function with MRI

MRI-derived parameters of LV diastolic function significantly improved after 3 months follow-up in bone marrow cell-treated patients as compared to placebo-treated patients (Table 4). Importantly, the increase in E/A peak flow ratio was significantly larger in the bone marrow cell group (p = 0.01), which was mainly based on an increase in the E peak flow rate, given that no improvement was observed in the A peak flow rate.

Table 4. Diastolic function (magnetic resonance imaging)

	BMC group		*Placebo group*		*Change*		*P*
	Baseline	*3 months*	*Baseline*	*3 months*	*BMC*	*Placebo*	
E/A peak flow	1.1±0.4	1.2±0.5	1.1±0.4	1.0±0.4	0.2±0.3	-0.0±0.2	0.01
E peak flow rate (mL/s)	407±96	468±110	421±127	391±116	61±89	-30±104	0.009
E peak flow rate/EDV (s^{-1})	2.3±0.6	2.7±0.8	2.4±0.7	2.3±0.8	0.4±0.6	-0.1±0.4	0.02
E acceleration peak (mL/s^2 x 10^{-3})	7.0±2.0	8.3±1.8	7.4±2.8	6.5±2.3	1.3±2.0	-0.9±2.0	0.002
E deceleration peak (mL/s^2 x 10^{-3})	-3.9±1.3	-4.8±1.9	-3.9±1.9	-3.5±1.6	-0.9±1.2	0.4±1.5	0.01
A peak flow rate (mL/s)	402±98	399±84	423±122	414±121	-3±73	-9±96	0.80
A peak flow rate/EDV (s^{-1})	2.3±0.8	2.3±0.8	2.5±0.8	2.5±0.8	0.0±0.4	0.0±0.5	0.89
A acceleration peak (mL/s^2 x 10^{-3})	7.7±2.5	7.5±2.2	7.4±2.4	7.7±2.2	-0.2±1.5	0.3±2.3	0.43
A deceleration peak (mL/s^2 x 10^{-3})	-6.3±2.3	-6.2±1.9	-6.0±2.5	-6.0±1.8	0.1±2.0	-0.0±2.5	0.81

EDV: End diastolic volume, BMC: bone marrow cell group

Discussion

The aim of the current study was to investigate the effect of intramyocardial bone marrow cell injection on LV diastolic function in patients with chronic myocardial ischemia. The present results demonstrate that bone marrow cell injection is associated with a modest but significant beneficial effect on both echocardiographic and MRI-derived parameters of LV diastolic function. The results of this study extend the previously reported observations that bone marrow cell injection improved myocardial perfusion, LV systolic function and anginal complaints in patients with chronic myocardial ischemia.[2]

Although several studies assessed the effect of intramyocardial bone marrow cell injection on myocardial perfusion and LV systolic function,[2,17] only limited data are available on the effect on diastolic function. In patients with acute myocardial infarction, an improvement in LV diastolic function was observed after intracoronary bone marrow cell infusion in a

substudy from the BOOST-trial,[3] which was partially sustained at 18 months follow-up but not at 5 year follow-up.[6] In a recent study using a pig model of chronic myocardial ischemia, Schneider et al. demonstrated that intramyocardial bone marrow cell injection with the use of the NOGA system could improve parameters of LV diastolic function.[5] Furthermore, in a nonrandomized clinical study from our group in patients with chronic myocardial ischemia, improved myocardial relaxation was observed after intramyocardial bone marrow cell injection.[8]

The current study is the first to confirm the beneficial effect of intramyocardial bone marrow cell injection on diastolic function in a randomized, double-blinded, placebo-controlled clinical trial in patients with chronic myocardial ischemia. In the present study, the E/E' ratio was chosen as a primary end-point of the study, since this parameter has been shown to provide a reliable estimate of LV filling pressures.[18] At baseline, patients had high-normal LV filling pressures and modestly impaired myocardial relaxation, as evidenced by an E' of 5.5 ± 1.4 in the bone marrow cell group and 5.6 ± 1.4 in the placebo group (p = 0.73). Filling pressure estimate E/E' showed a modest but significant improvement in the bone marrow cell group as compared to the placebo group. The improvement in filling pressure was confirmed by a decrease in speckle tracking-derived filling pressure estimate $E/GSR_{ivr.}$ Filling pressures are mainly determined by volume load on one side and ventricular compliance and myocardial relaxation on the other side.[18] Since myocardial relaxation improved, as indicated by E', diastolic strain rate and MRI-derived flow measurements, it is conceivable that the decrease in filling pressure is mainly related to the improvement in myocardial relaxation. Of note, parameters of ventricular compliance, such as deceleration time and peak atrial filling did not reveal any changes in ventricular compliance. Although filling pressure estimates were decreased at 3 months follow-up, no decrease in left atrial size was observed. This may be related to the short follow-up period, which is probably too short to observe structural changes of the left atrium. Nonetheless, it must be noted that the observed improvements in filling pressure estimates may be too modest to be accompanied by structural changes in the left atrium. Diastolic dysfunction, and impaired myocardial relaxation in particular, has been established as an early consequence of myocardial ischemia.[19,20] All patients included in the current study had chronic myocardial ischemia as documented using SPECT, caused by

severe coronary artery disease. Hence, it is likely that the modestly impaired myocardial relaxation at baseline, resulting in high-normal filling pressures, is a consequence of the presence of myocardial ischemia. Experimental studies have demonstrated that bone marrow cell injection may enhance neovascularization through secretion of pro-angiogenic cytokines and physical incorporation of the injected cells into new capillaries or in perivascular cells.[21,22] In line with these observations, the present trial documented an improvement in myocardial perfusion and a reduction in myocardial ischemia after intramyocardial bone marrow cell injection.[2] In patients undergoing coronary artery bypass grafting, improvements in myocardial relaxation and filling pressures were reported by Carluccio et al.[23] Accordingly, it may be hypothesized that the observed improvements in myocardial relaxation and, subsequently, filling pressures are secondary to enhanced myocardial perfusion. From a mechanistical point of view, the current data support this hypothesis, since the enhanced myocardial perfusion would be expected to improve active processes such as myocardial relaxation rather than altering myocardial structure and ventricular compliance. The effect of bone marrow cell injection on LV diastolic function was evaluated using both MRI and echocardiography. Phase contrast flow velocity mapping by MRI is a well-validated automated technique for the evaluation of diastolic function.[24,25] By measuring flow-velocity as well as flow-volumes across the mitral valve orifice, it allows reliable measurement of peak early filling, peak atrial contribution to filling, and the end of filling.

In the present study, flow velocity mapping detected an increase in the E/A peak flow ratio, indicating improved myocardial relaxation. In the present study, evaluation of regional myocardial function using speckle tracking strain analysis documented significant improvements in global strain and strain rate after bone marrow cell injection. In a multivariable linear regression model, no significant interaction was observed between treatment allocation and improvement in segmental strain and strain rate, respectively. Nonetheless, within-group analysis revealed that the improvement in LV systolic function in bone marrow cell-treated patients is predominantly caused by enhanced myocardial function in the injected myocardial segments. This observation is in line with the results from the study of Herbots et al. in patients with acute myocardial infarction, which demonstrated improved recovery of regional function in the infarct-related segments after bone marrow cell transfusion in the infarct-related artery.[26] Of note, the patients in the

present study were not recovering from acute myocardial infarction, but had stable coronary artery disease resulting in chronic myocardial ischemia. Therefore, this observation suggests that bone marrow cells can not only stimulate an ongoing recovery process after myocardial infarction, but may also initiate focal improvement in a stable situation such as chronic myocardial ischemia. This might be regarded as an encouraging finding with regard to cell-based treatment strategies for stable angina, ischemic heart failure or non-ischemic cardiomyopathy. In addition, the regional effect observed in the injected segments suggests that the amount of cells that is retained in the myocardium is apparently sufficient to induce these objective improvements in regional function, which is an important observation with regard to the doubts that have been raised with regard to the engraftment rate and cell survival after injection.[27] The results of the present study have several implications. Importantly, this study demonstrates that the functional benefit of bone marrow cell injection is not confined to myocardial perfusion and systolic function. Although only modest improvements in diastolic function were observed, the effects on diastolic function may be considered in the design of future studies. In addition, because diastolic dysfunction is a prognostic factor, future studies have to evaluate whether the improvement in diastolic function is related to better outcome in these patients with severe coronary artery disease. The present study has several limitations. First, a minority of the patients included in the randomized trial was not available for inclusion in the current substudy due to atrial fibrillation, the inability to undergo MRI scanning, or insufficient flow measurements quality. However, the baseline characteristics of the patients in this substudy were comparable with those of the entire study cohort. Second, the follow-up period of the present study is relatively short to monitor changes in diastolic function, since changes in ventricular compliance or left atrial volume may be evident after a longer follow-up period. In addition, in the current design it is not possible to document whether the improvement in diastolic function will be sustained over time after 3 months follow-up. Finally, invasive measurements were not used in the current study and diastolic function was evaluated using noninvasive estimates of diastolic function.

Conclusions

The current substudy of the randomized, double-blinded, placebo-controlled trial from our group demonstrates that intramyocardial injection of autologous bone marrow-derived mononuclear cells is associated with a beneficial effect on both MRI- and TDI-derived parameters of diastolic function in patients with chronic myocardial ischemia.

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