Intracoronary infusion of selected autologous bone marrow stem cells improves longitudinal myocardial strain and strain rate in patients with old anterior myocardial infarction without recent revascularization

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Aims
We sought to evaluate the efficacy of intracoronary infusion of selected bone marrow stem cells (BMSCs) in patients with remote, anterior non-viable MI by the use of tissue Doppler imaging.

Methods and results
We infused selected CD133+ and CD133-CD34+ BMSCs in 10 patients enrolled in the study. Peak systolic strain rate, maximum strain during the cardiac cycle ($e_{\text{max}}$), strain during ejection time ($e_{\text{et}}$), and post-systolic strain ($e_{\text{ps}}$) were measured. Peak systolic strain rate ($-0.69 \pm 0.2$ vs. $-1.15 \pm 0.27, P = 0.001$), $e_{\text{max}}$ ($-9.87 \pm 3.30$ vs. $-15.57 \pm 5, P = 0.006$), and $e_{\text{et}}$ ($-7.45 \pm 2.86$ vs. $-10.92 \pm 4.45, P = 0.015$) improved significantly during the rest study 6 months after cell infusion. Low-dose inotropic challenge also showed significant improvement of longitudinal deformation indices in the follow-up study. Global ejection fraction did not improve significantly after cell therapy.

Conclusion
Intracoronary infusion of selected BMSCs in patients with remote, anterior, non-viable myocardial infarction is safe and leads to improvement of longitudinal deformation indices 6 months after the infusion.

Keywords
Selected bone marrow stem cells • Intracoronary infusion • Anterior wall myocardial infarction • Longitudinal deformation indices

Introduction
Experimental data suggest that transfer of stem cells and bone marrow-derived progenitor cells enhances cardiac performance early after an acute myocardial infarction. Several mechanisms including infused stem cell plasticity and differentiation to myocardial cells, neoangiogenesis through stem cell adherence in coronary vessel walls, cell attraction, fusion and accumulation through chemotacticity, paracrine mechanisms, and modification of the scar properties leading to preservation of left ventricular function have been proposed to explain these experimental beneficial effects.1–6

Clinical studies have indicated that intracoronary infusion of autologous bone marrow stem cells (BMSCs) in the infarcted zone 4–18 days after an acute myocardial infarction promoted global and regional functional recovery, while others found only temporary or no benefit. These results pertain only to patients with recent myocardial infarction in the setting of percutaneous revascularization.7–14 Scarce previous attempts have been made to use stem cells and evaluate the efficacy of this therapy in
patients with non-viable, post-infarction scarred myocardium using skeletal myoblasts mostly combined with reperfusion by coronary artery bypass grafting.15–20 Strauer et al.21 reported favourable effects of intracoronary delivery of bone marrow-derived mononuclear cells (BM-MNCs) on functional and metabolic regeneration of chronic non-viable infarcted myocardium. Our group reported recently a significant benefit in terms of regional perfusion, global function, and remodelling using bone marrow derived CD133+ and CD133-CD34+ stem cells in patients with heart failure due to ischemic cardiomyopathy.22,23

Conventional two-dimensional (2D) echocardiographic analysis of regional wall motion may not be suitable to identify the small changes that have been documented after cell therapy. Recovery of myocardial function after an ischaemic event or during inotropic stimulation, indicating myocardial viability, may occur below the threshold of visual detection.24 The differential thickening of myocardial layers, fibre orientation, and tethering with adjacent healthy myocardial segments may also obscure wall motion analysis by 2D echocardiography.25 Assessment of longitudinal systolic myocardial strain (ε) and strain rate (SR) by tissue Doppler imaging (TDI) offers an objective and sensitive means to quantify small changes in regional left ventricular function.26–28 This method has not been previously reported in the evaluation of cell therapy. Our study was undertaken to assess the effect of intracoronary infusion of selected BMSCs in patients with remote MI without evidence of viability by the use of the TDI technique.

Methods

Ten patients (nine males) aged 43–62 years were included in the study. Patients were enrolled on the basis of the following inclusion criteria: (i) history of an old, transmural, anterior myocardial infarction, (ii) impaired left ventricular function with ejection fraction at baseline below 35%, (iii) absence of viability of the anteroapical segments assessed by Tl-201 reinjection scintigraphy and low-dose 2D dobutamine stress echocardiography, and (iv) known patency of the left anterior descending coronary artery (LAD). The exclusion criteria were: history of coronary artery bypass grafting, percutaneous coronary intervention for intracoronary infusion.

Conventional two-chamber view was used, and the flow signal was located by colour flow mapping as flow towards the transducer containing a dominant diastolic signal.31,32 Analysis of TDI images was performed blindly by two independent observers. The wall motion was tracked manually and care was taken to maintain a fixed mid-wall position, avoiding signals from the left ventricular cavity. Deformation curves were obtained from the anteroapical region of the LAD territory. Two heart cycles were used to average deformation curves. Event timing of aortic and mitral valve opening and closure was obtained as described previously.33 Peak systolic strain rate (PSSR), maximum strain during the cardiac cycle (εmax), strain during ejection time (εe) and post-systolic strain (εp), defined as myocardial shortening occurring after aortic valve closure and before the onset of myocardial lengthening, were measured.26–28 Furthermore, ejection time strain was measured from five adjacent sites of the anteroapical and five sites of the lateral wall. The average ejection time strain of these two walls before BMSC infusion was compared with the average strain of the same walls after cell therapy.

Bone marrow harvest and stem cell separation

Bone marrow harvesting and cell processing have been described previously.23 Bone marrow (310 ± 40 mL) was harvested from the posterior iliac crest under local anaesthesia. The collected bone marrow was immediately transferred to the haematology laboratory where two types of bone marrow cells were separated and isolated under sterile conditions. The first population was cells bearing the CD34 surface marker that is expressed mainly by haemopoietic stem cells, while the other one were CD133+ cells, a more immature population of progenitor cells with multiple differentiation potential. The isolation was performed using the MACS immunomagnetic method (Miltenyi Biotech, Bergisch-Gladbach, Germany) as described elsewhere. Briefly, BM-MNCs were separated by Ficol density centrifugation and were subsequently incubated for 30 min at 4°C with a monoclonal antibody conjugated with magnetic beads against stem cell marker CD133. After a single wash, coupled cells were passed through an LC-MACS separation column (Miltenyi, Biotech) held within a magnetic field to retain CD133+ cells that were subsequently eluted after the column was removed from the magnet. Unbound CD133-negative cells were washed out and re-incubated with an anti-CD34 antibody (Miltenyi, Biotech), and by the same immunomagnetic procedure, CD133-CD34+ progenitor cells were also isolated. After this double isolation procedure, specimens were taken for enumeration and quality control (microbial cultures, recovery, viability, purity, and functional analysis), and finally a total of 1.6 × 10^7 ± 0.5 progenitor cells were collected for each patient, diluted in 10 mL of human albumin 5% and sent back to the cardiac catheterization laboratory for intracoronary infusion.

Intracoronary stem cell infusion

Intracoronary infusion of the isolated BMSCs was performed 6 ± 2 h after bone marrow harvest. Bone marrow stem cells were infused through an over-the-wire balloon catheter at the proximal portion
of the LAD. During BMSC infusion, the balloon was inflated for 5 ± 2 min to facilitate cell homing and vascular adherence.

**Statistical analysis**

Values are expressed as mean ± 1 SD. Comparisons of echocardiographic and deformation indices before and 6 months after BMSC infusion were performed by the Wilcoxon test for paired observations. Differences in TDI indices at rest and during dobutamine infusion were evaluated by the Wilcoxon test. Correlations between measurements were assessed using the Spearman rho coefficient. To test the reproducibility of the measurements, two similarly and well-trained physicians measured in the same patient various echocardiographic and deformation indices. The Bland and Altman method was applied to evaluate the reproducibility of all echocardiographic and deformation measurements and to assess any potential systematic bias. All statistical analyses were performed in SPSS version 12 statistical software. A P-value < 0.05 for two-sided hypotheses was considered as statistically significant.

**Results**

**Reproducibility**

By calculating the rho correlation coefficient between the average and difference of the investigated echocardiographic and deformation measurements of the two observers, we found that the values of rho varied from −0.071 to 0.396 (P > 0.38). Moreover, mean differences of these indices were within the 95% confidence limits. These indicate no systematic, significant bias in all the investigated measurements.

**Baseline characteristics**

Baseline characteristics of this patient’s cohort are shown in Table 1. The iliac crest puncture, BMSC aspiration, and the progenitor cell infusion procedure were well tolerated, with the exception of one vasovagal attack during iliac crest puncture that resumed with intravenous administration of fluids and atropine. No other major events occurred during the procedure. All patients were alive in stable condition during 12–22 months after BMSC infusion.

No evidence of malignant arrhythmias was noted during the procedure or at 24 h Holter monitoring during follow-up. Low-dose dobutamine echocardiography was also well tolerated without 2D echocardiographic evidence of ischaemia or viability.

Echocardiographic detection of left anterior descending flow by transthoracic echocardiography prior to BMSC infusion and quantitative analysis of deformation were possible in all patients.

**Effect of cell therapy**

The effect of BMSC infusion at rest in the 6-month follow-up study is summarized in Table 2. No significant change was noted in left ventricular ejection fraction. Systolic longitudinal deformation indices (ε_{max}, ε_{ps} and PSySR) improved significantly 6 months after intracoronary BMSC implantation, whereas ε_{ps} showed no significant change. We also compared the average ejection time strain derived from five adjacent sites of the anteroseptal wall with the average strain of five adjacent sites of the normal, lateral wall (Figure 1). The average ejection time strain and PSySR of the anterior wall during the rest study and after dobutamine infusion increased significantly after BMSC infusion, whereas these two longitudinal deformation indices remained unchanged 6 months after cell therapy (Tables 3 and 4).
Before BMSC treatment, left ventricular ejection fraction at rest was 27.5 ± 6.4% and increased to 31.8 ± 9.6% (P = 0.016) with Dobutamine, while \( e_{\text{a}} \) \(-7.45 \pm 2.9\) to \(-9.59 \pm 3.72\), \(P=0.032\), \(e_{\max} \) \(-9.87 \pm 5.3\) to \(-9.85 \pm 4.6\), \(P=0.019\), and \( \Delta e_{\text{a}} \) \(-0.69 \pm 0.21\) to \(-0.93 \pm 0.23\), \(P=0.005\) also improved significantly. After BMSC infusion, only left ventricular ejection fraction \((30 \pm 7.6 \text{ to } 33.7 \pm 8.7\% \text{ } P=0.024\)) and \(e_{\max} \) \((-13.6 \pm 4.9\text{ to }-17.93 \pm 4.86\text{, }P=0.007\)) improved significantly during inotropic challenge. Dobutamine infusion had no significant effect on WMSI or \(e_{\text{a}}\) either before or after cell therapy.

### Discussion

Our study evaluates the effect of intracoronary infusion of selected autologous bone marrow stem/progenitor cells on regional and global function recovery in patients with remote, non-viable anterior myocardial infarction. We injected a large amount of BMSCs into the proximal part of the LAD. This infusion produced a significant improvement in regional systolic function documented by local deformation echocardiographic indices. The procedure was safe and feasible; however, the clinical efficacy could not be documented owing to the small size of the patients treated. The main difference to most previous studies is that we selected patients with old, non-viable myocardial infarction who did not undergo any revascularization procedure prior to cell infusion. In such patients, myocardial damage is considered 'irreversible' and the remodelling process complete. Most studies emphasize the importance of BMSC injection to a region with viable myocardium. In a clinical and therapeutic level, this approach led most investigators to select patients with recent reperfused MI.7,9 However, detection of myocardial viability remains an unresolved issue for cardiology practice. In fact, we failed to demonstrate viability by Tl201 scintigraphy and dobutamine 2D echocardiography, but longitudinal deformation indices improved at rest as well as after dobutamine infusion.

Strain derives from myocardial velocity assessed by TDI. Longitudinal systolic strain is the shortening between two adjacent myocardial segments during systole. Strain rate is the time derivative of strain. Experimental data suggest that strain is closer related to sonomicrometry-derived local deformation than velocity indices.27 Clinically, strain is more accurate than 2D systolic wall thickening analysis to depict regional wall motion abnormalities in post-infarction patients.35

In the study of Hoffmann et al.,25 it was demonstrated, by comparison with18 FDG-PET, that an increase in systolic strain rate by \(-0.23\)\% produced by dobutamine infusion is superior to dobutamine stress 2D echocardiography and TDI in predicting myocardial viability.

The improvement of PSySR was the most consistent and pronounced effect of BMSC infusion in our study. Peak systolic strain rate increased in our study by 67% at rest and 76% when combined with dobutamine infusion 6 months after BMSC infusion. Ejection time strain and maximum strain exhibited smaller changes in our study. Post-systolic strain remained unchanged. This lack of change in post-systolic strain could be attributed to the fact that this index is considered an indicator of ischaemia rather than contractile reserve.

Despite the lack of viability on 2D regional wall motion visual analysis, low-dose dobutamine infusion produced an increase in apical deformation indices during the initial study which was performed prior to BMSC infusion. Six months after BMSC treatment, the effect of dobutamine infusion was attenuated, producing only an increase in apical \(e_{\max}\). Values of anteroapical deformation indices at rest 6 months after cell therapy were similar to those during dobutamine infusion before therapy.

No previous study has used local deformation to evaluate the effect of BMSC infusion in patients with remote, non-viable myocardial infarction. Magnetic resonance was used in the BOOST...
trial to prove an increase in global and regional functions in patients with recent myocardial infarction who underwent intracoronary BMSC infusion.

We selected to isolate CD133+ and CD133-CD34+ stem/progenitor cells because these cells are able to differentiate into endothelial, mesenchymal, and haemopoietic cells. Stamm et al. reported improvement of regional function and local perfusion following intramyocardial injection of CD133+ cells during CABG in patients with myocardial infarction at 4 weeks prior to surgery. More recent experimental data suggest that CD133+ injection in the myocardial scar of an animal model results in significant functional improvement of this ‘permanently’ damaged myocardial region. An alternative approach would be to inject unselected cells or even total bone marrow in order to avoid the significant cost of specific cell separation. The development of microinfarctions or severe myocardial calcification, however, may complicate such an approach.

Limitations of the study
The small number of patients and the absence of a control group limit the value of the study conclusions. We used the lateral wall as ‘control’ in the sense that no cell infusion was made in this wall. Furthermore, the length of follow-up does not permit any conclusions about the potential long-term benefit of cell therapy. Several reports show that the initial recovery of global ventricular function after intracoronary infusion of autologous BM-MNCs attenuated after a follow-up time of 18 months.

Conclusion
In our study, we included patients with a patent LAD coronary artery and no viability in the anterograde region by the classical methods. The infusion of BMSCs in the LAD improved the longitudinal left ventricular function of the infarcted anterograde wall assessed by TDI 6 months after therapy.

Conflict of interest: none declared.

References


