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Does Transendocardial Injection of Mesenchymal Stem Cells Improve Myocardial Function Locally or Globally? An Analysis From the POSEIDON Randomized Trial

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Abstract

Rationale—Transendocardial Stem Cell Injection (TESI) with mesenchymal stem cells improves remodeling in chronic ischemic cardiomyopathy, but the impact of the injection site remains unknown.

Objective—To address whether TESI exerts its effects at the site of injection only or also in remote areas, we hypothesized that segmental myocardial scar and segmental ejection fraction improve to a greater extent in injected than in non-injected segments.

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DISCLOSURES

Dr Hare reported having a patent for cardiac cell-based therapy, receiving research support from Biocardia, being a board-member, consultant, and having equity interest in Vestion Inc, and being a consultant for Kardia.

Dr. Joshua Hare holds equity in Vestion and maintains a professional relationship with Vestion as a consultant and member of the Board of Directors and Scientific Advisory Board. Dr George reported serving on the board of GE-Healthcare, consulting for ICON-Medical-Imaging, and receiving trademark royalties for fluoroperfusion-imaging. Mr Mendizabal is an employee of EMMES-Corporation. Dr Altman is employee of Biocardia-Inc. Dr McNiece reported being a consultant and board member of Proteonomic-Inc. Dr Heldman reported having a patent for cardiac cell-based therapy, receiving research support from Biocardia, and being a board-member, consultant, and having equity interest in Vestion Inc. No other authors reported any financial disclosures.

Methods and Results—Biplane ventriculographic and endocardial tracings were recorded. TESI was guided to 10 sites in infarct-border zones. Sites were mapped according to the 17-myocardial segment model. As a result, 510 segments were analyzed in 30 patients before and 13-months after TESI. Segmental early enhancement defect (SEED, a measure of scar size) was reduced by TESI in both injected ($-43.7\pm 4.4\%$, $n=95$, $p<0.01$) and non-injected segments ($-25.1\pm 7.8\%$, $n=148$, $p<0.001$; between group comparison $p<0.05$). Conversely, segmental ejection fraction (SEF, a measure of contractility) improved in injected scar segments (19.9 ± 3.3 to $26.3\pm 3.5\%$, $p=0.003$) but not in non-injected scar segments (21.3 ± 2.6 to $23.5\pm 3.2\%$, $p=0.20$, between group comparison $p<0.05$). In the subgroup of scar segments with baseline SEF $<20\%$, the SEF improvement was even greater in injected segments ($12.1\pm 1.2\%$ to $19.9\pm 2.7\%$, $n=18$, $p=0.003$) vs. non-injected segments ($13.3\pm 1.3\%$ to $16.1\pm 2.1\%$, $n=15$, $p=0.05$; between group comparison $p<0.05$).

Conclusions—These findings illustrate a dichotomy in regional responses to TESI. Although scar reduction was evident at the site of TESI and remotely, ventricular functional responses occurred preferentially at the sites of TESI. Furthermore, improvement was greatest when segmental left ventricular dysfunction was severe.

Keywords

Myocardial infarction; imaging; tomography; cells

INTRODUCTION

Although there are accumulating preclinical^{1, 2} and clinical trial^{3, 4} data supporting the use of transendocardial stem cell injection (TESI)⁵⁻⁹ to produce reverse remodeling in chronic heart failure, the impact of injection site is unknown. In the Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON) trial⁴, TESI with autologous or allogeneic mesenchymal stem cells (MSCs) delivered to 10 sites around the infarct border zone improved left ventricular (LV) structure and function globally and resulted in reduced scar size, reduced LV volumes, and restored LV sphericity index toward normal⁴. The mechanistic basis underlying the myocardial regenerative effect(s) of MSCs include both direct^{2, 10, 11} and paracrine actions^{8, 12}. Whether these actions are exerted locally, at a distance, or globally, and the impact of precisely localizing cell injections remain unclear. The POSEIDON trial⁴ results indicated improved global ventricular structure and function, but regional effects might be obscured by conventional LV imaging analysis¹³.

Here we combined the imaging advantages of Multidetector computed tomography (MDCT) and biplane left ventriculography to perform a myocardial segmental analysis of the POSEIDON clinical trial so as to test the hypothesis that sites of cell injection respond more favorably in terms of cardiac repair than sites not receiving cell injections. We investigated whether injected myocardial segments have greater reduction of segmental early enhancement defect (SEED, an indicator of myocardial scar) and improved ventricular performance, measured by segmental ejection fraction (SEF, a measure of regional myocardial contraction) in comparison to infarcted non-injected myocardial segments. The findings of this study have important implications for implementing stem cell therapy delivered by transendocardial injection.

METHODS

A full description of the study protocol, inclusion and exclusion criteria has been published⁴. All patients provided written informed consent for the University of Miami Institutional Review Board–approved protocol; enrollment and exclusions are shown in Online Figure I. In summary, POSEIDON was a phase I/II randomized, open-label clinical trial, designed (1) to explore the safety of allogeneic MSCs and (2) to compare the long-term safety and efficacy of allogeneic MSCs with autologous MSCs in patients with chronic LV dysfunction secondary to myocardial infarction (Baseline characteristics shown in Online Table I). Our earlier publication⁴ reported clinical safety and efficacy. Here, we used the POSEIDON imaging data and the total population of POSEIDON, to explore mechanistic insights related to the site of the injection.

MDCT analysis

MDCT provides detailed and accurate information that is useful in the preparation of an injection strategy, and in follow-up examination permits quantifying the response to stem cell therapy¹⁴. Cineangiographic MDCT was used for reconstruction of images in 30 patients at screening and at 13-months after TESI for analysis of scar size, segmental ejection fraction and wall thickening. Acquisition of images was performed using 128 slice (Siemens AS+, Siemens Medical Solutions) or 320-slice (Aquillion One-Toshiba) CT scanning systems with a spatial resolution of 0.30mm and 350 microns respectively, and analyzed using iNtuition version 4.4.7.47 (TeraRecon, Inc., Foster City, Calif., USA) as described previously⁴.

We evaluated the regional response to MSCs by measuring myocardial SEED as an indicator of myocardial scar size and SEF as a measure of regional myocardial contractility. Within regards to scar size, delayed enhancement (DE) obtained with cardiac magnetic resonance (CMR) imaging is often used to measure scar size; however, MDCT derived DE and early enhancement defect (EED) has also been used in studies to quantify scar size^{4, 15-17}. Schueleiri et al.¹⁴ described cardiac MDCT-DE in a swine model and concluded that it can accurately quantify scar tissue for preclinical and clinical studies for novel myocardial therapies. Mahnken et al.¹⁷ compared DE-CMR, DE-MDCT, and EED-MDCT in 28 patients within 2 weeks after MI and reported strong correlation between DE-CMR and DE-MDCT. DE-CMR and EED-MDCT were tightly correlated, they found that EED-MDCT underestimates the size of MI (mean size of MI was 31.2g on DE-CMR, 33g on DE-MDCT, and 24.5g on EED-MDCT)¹⁷. Nevertheless, MDCT-derived SEED analysis, as described in our earlier publication⁴, was preferred as a marker of infarct size since it provided 1) discernible difference in densities between normal and abnormal (infarcted) myocardium, 2) better demarcation of the endocardial border of abnormal myocardium in comparison to DE-MDCT, and 3) better image quality and less susceptibility to beam hardening artifacts secondary to automatic implantable cardioverter-defibrillator leads, which were implanted in all but one of our patients.

For regional analysis of myocardial function, previous studies¹⁸, using electron beam computed tomography (EBCT), demonstrated that SEF can detect abnormalities and provide accurate identification of ischemic myocardium in patients with varying degrees of coronary

artery occlusion. Similarly, by applying the principle of SEF to regenerative myocardial therapy, we have been able to analyze regional myocardial performance and scarring following TESI in patients with chronic ischemic cardiomyopathy (Online Figure II).

Myocardial infarct scar assessment: SEED

Assessment of myocardial SEED was previously described^{4, 15, 16}. Briefly, contiguous 8 mm thick short-axis reconstructions were made for the end diastolic, end systolic, as well as mid diastolic (usually 70% of the R-R interval) cardiac phases encompassing the entire left ventricular myocardial volume. Normal reference CT based myocardial density was measured (Hounsfield units – HU) from regions of interest of approximately 10-15 mm² in a myocardial segment that demonstrated normal regional function, i.e. normal myocardial wall motion and thickening. For optimal evaluation of myocardial SEED, the CT window level was adjusted so that the center of the scale corresponded to 20 HU below the normal myocardial density reference value^{17, 19}. This approach was chosen to define a visual threshold of approximately 2 standard deviations below the mean of normal myocardium enhancement, allowing optimal delineation of hypoenhancement. A narrow window width was used (100–150 HU) to emphasize the threshold between normal and abnormal myocardium and still allow the surrounding anatomy to be visualized. SEEDs were identified by their crescentic shape, subendocardial and/or transmural location, and presence in at least two of the three examined cardiac phases. Total myocardial SEED mass per patient was calculated by summing all segmental defect areas and multiplying the total by section thickness (0.8 cm) and then by 1.05 g/cm³ (myocardial specific gravity)¹⁹. Each SEED identified in each short-axis section (4 slices at base, 4 slices at mid, and distal 3-4 slices at apical LV regions) was allocated into either six (base and mid LV regions) or four (apical LV region) myocardial segments according to the AHA 17-myocardial segment model²⁰.

EF Analysis: Global EF analysis and SEF analysis

Cardiac volumes and global LVEF ((LV End Diastolic Volume - LV End Systolic Volume) * 100) / LV End Diastolic Volume) were calculated, and 17 segment polar maps were generated. SEF is an expression of regional function, reflecting the volume of blood ejected under a given myocardial segment, contributing to the global LVEF¹⁸. The left anterior descending (LAD) artery in the interventricular sulcus between the basal anterior (AHA seg #1) and the basal anteroseptal (AHA seg #2) segments in the short axis view was used as an anatomical landmark for segmentation. The basal edge of the LV was identified by the mitral valve annulus in 4 and 2 chamber views. Definition of endocardial borders of the LV chamber in end-systolic and end-diastolic phases were manually assessed with contour corrections, when necessary, following the software protocol recommendations.

For SEF calculation, the software assumes that each one of the 17-AHA segments is composed from numerous fan shaped micro-segments (Online Figure II). The volume of all micro-segments is obtained in end-diastole and end-systole while maintaining an automated-independent center of the LV in the endsystolic phase, so called “fixed axis”. The individual SEF value presented in the polar map for all 17-AHA segments represents the average SEF of all fan shaped micro-segments within the same particular segment ((Segmental LV End

Diastolic Volume – Segmental LV End Systolic Volume)*100) / Segmental LV End Diastolic Volume)..

Wall thickening

Segmental wall thickening (Systolic wall thickness- Diastolic wall thickness) was calculated following the 17-segments model for each patient.

Topographic analysis

We further evaluated the regional response to MSCs by conducting topographic analyses using MDCTEED images. To address whether there was a similar response (SEED and SEF) in transmural vs. nontransmural scars all scars were grouped based on baseline transmurality (>50% and <50%).

Strategy of cell delivery in the POSEIDON trial

Catheter left ventriculography guided the sequence of injections, and was compared with MDCT for procedural planning and selection of target sites. Biplane left ventriculography was performed in the 60° LAO and 30° RAO views⁵. MDCT global and regional cardiac function permitted for the assessment of myocardial viability and wall motion defects. Together these orthogonal projections describe the position and contractility of each myocardial segment. End-diastolic endocardial contour tracings were traced from these images; hypokinetic and akinetic segments were marked as infarct zone and the adjacent area to it was identified as the border zone. During TESI each site of injection was recorded onto these ventriculographic maps in two projections.

17-segment model reconstruction: Biplane left ventriculography- Endocardial tracings and use of the 17-segment model for recognition of areas injected

Injection sites were manually translated onto 17-segment polar maps by two independent cardiologists (JPZ and KN), who assessed if a segment received at least one injection. Overall agreement between the two readers resulted in a Kappa coefficient of 0.8896 (95% CI, 0.8447-0.9346; p<0.0001). Disagreement was resolved by a third reader (VK). All readers were blinded to the SEED and SEF values. Each injection site was allocated to one of the 17 segments (Figure 1).

Definition of subgroups of myocardial segments for analysis

For segmental analysis, myocardial segments were categorized as follows; “Scar- injected” included all myocardial segments with SEED treated with TESI. “Scar-non-injected” included all myocardial segments with SEED not treated with TESI. “Non-scar-injected” included all myocardial segments without SEED treated with TESI. And “non-scar-non-injected” included all myocardial segments without SEED not treated with TESI. Additionally, for radius of activity analysis purposes, the segments around and in the opposite side of the “scar-injected” were categorized as “adjacent” and “remote” myocardial segments respectively. Within each patient, we compared the accumulated mass value of all SEED treated and non treated with TESI before and after TESI. Finally, to elucidate the changes in each subgroup of segments, we correlated the polar maps obtained from MDCT

with those marking injection sites to perform a myocardial segmental analysis of the POSEIDON clinical trial.

Statistical analysis

For analysis purposes, all 30 patients who received either autologous or allogeneic MSCs were assessed. All data are presented as mean \pm standard error of mean. GraphPad Prism (Version 4.03, La Jolla, CA) was used to analyze all data points. To compare the change in SEF and SEED at 13-month follow-up, paired t-tests were applied. For between group comparisons, one and two way ANOVA were applied with a Bonferroni/Dunn's Multiple comparison test when applicable. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Baseline scar characteristics

The baseline scar characteristics in the “scar-injected” and “scar-non-injected” groups were not significantly different with respect to scar size or thickness of segment with scar; 83.3% (n=25) and 96.7% (n=29) of myocardial segments were transmural in the “scar-injected” and “scar-non-injected,” respectively (Online Table II).

Greater scar size reduction at sites of stem cell injection

Similar scar size reduction was observed in the “scar-injected” segments with autologous (from 10.2 ± 1.8 g to 5.5 ± 0.8 g, n=15, p=0.001) or allogeneic MSCs (from 9.6 ± 1.7 g to 5.3 ± 1.1 g, n=15, p=0.001). The “scar-non-injected” segments had similar reduction in both groups (from 13.2 ± 2.5 g to 9.5 ± 1.7 g, p<0.01 for the autologous and from 10.1 ± 1.5 g to 7.3 ± 1.2 g p=0.002 for the allogeneic group). When considering the autologous and allogeneic groups combined, the scar size was reduced by $-43.7 \pm 4.4\%$ (from 9.8 ± 1.2 g to 5.4 ± 0.7 g, n=30, p<0.01) in the “scar-injected” vs by $-25.1 \pm 7.8\%$ in the “scar-non-injected” segments (from 11.7 ± 1.4 g to 8.6 ± 1.0 g, n=30, p<0.001; between group comparison “scar-injected” vs. “scar-non-injected” p<0.05; Figures 2, 3, and Online Video II). When considering cell type and cell dose, the greatest reduction was found in the injected segments that received 20 million autologous MSCs (from 7.1 ± 1.4 g to 3.7 ± 0.8 g p=0.02, between group comparison p<0.01, Online figure III).

Segmental EF improves in injected segments, but is unchanged in non-injected segments

A total of 510 myocardial segments were analyzed in a total of 30 patients. Improvement in myocardial function was observed in “scar-injected” segments in both the autologous (from $19.0 \pm 3.0\%$ to $25.25 \pm 3.2\%$, n=15, p=0.03) and allogeneic groups (from $20.9 \pm 3.7\%$ to $27.22 \pm 3.9\%$, n=15, p=0.02; between group comparison p=NS). When considering the autologous and allogeneic groups combined, 95 myocardial “scar-injected” segments were evaluated in a total of 30 patients; SEF improved by $+44.3\% \pm 11.2\%$ (from $19.9 \pm 3.3\%$ at screening to $26.3 \pm 3.5\%$) at 13-months after TESI (p=0.003). Conversely, SEF did not significantly improve when 148 “scar-non-injected” segments were evaluated alone or combined. The “non-scar-injected” (n=63) and “non-scar-non-injected” (n=204) myocardial segments did not demonstrate changes in SEF at 13-months (Figure 4). There was no

difference between groups when considering cell type and cell dose. Wall thickening significantly improved in the “scar-injected” segments ($1.6\pm 0.34\text{mm}$ at baseline to $2.2\pm 0.38\text{mm}$ at 13-months, $n=30$, $p=0.02$) but not in the “non-injected segments” ($1.5\pm 0.24\text{mm}$ at baseline to $2.0\pm 0.37\text{mm}$ at 13-months, $n=30$, $p=0.2$), between groups $p>0.05$. In addition, the Pearson correlation coefficient between the number of “scar-injected” segments and the improvement of myocardial SEF was $r=0.57$ (95% CI, 0.23 to 0.78; $p=0.002$; Figure 6). There was no difference between groups when considering cell type and cell dose.

Radius of activity as measured by SEF

From our earlier analysis, SEF improved by $+44.3\pm 11.2\%$ (from $19.9\pm 3.3\%$ at screening to $26.3\pm 3.5\%$) at 13-months after TESI ($p=0.003$) in all “scar-injected” segments. To analyze the radius of activity, segments adjacent to and remote from “scar-injected” segments were compared. We observed that SEF improved in “adjacent” segments in both the autologous (from $18.9\pm 1.7\%$ to $26.0\pm 2.8\%$, $n=15$, $p=0.005$) and allogeneic groups (from $29.4\pm 4.3\%$ to $34.0\pm 4.0\%$, $n=15$, $p=0.02$; between group comparison $p>0.05$). When autologous and allogeneic groups were combined, SEF % change from baseline improvement in “adjacent” segments was $+27.8\pm 6.4\%$ (from $24.2\pm 2.6\%$ to $30.1\pm 2.6\%$, $n=30$, $p=0.001$). On the other hand, the “remote” segments did not demonstrate changes in contractility when considering cell type and cell dose, nor when combining autologous and allogeneic groups (% change in SEF from baseline was $10.1\pm 8.9\%$ from $25.8\pm 3.2\%$ to $27.0\pm 3.6\%$, $n=30$, $p=0.4$). Importantly, SEF in “scar-injected” and “adjacent” segments improved more than “remote” segments (one way analysis of variance $p=0.003$. Dunn's post test $p<0.05$ in “scar-injected” vs. “remote” and “adjacent” vs. “remote”; Figure 5)

Improvement in contractility is particularly evident in the highly dysfunctional segments

In a subgroup analysis of all segments with a SEF $<20\%$, the mean SEF in “scar injected” myocardial segments was $12.1\pm 1.23\%$ at baseline and improved to $19.9\pm 2.69\%$ ($p=0.003$) at 13-months after TESI ($n=18$ patients). In this subgroup analysis, the “scar non-injected” myocardial segments ($n=15$ patients) showed a trend of increase SEF from $13.3\pm 1.3\%$ to $16.1\pm 2.13\%$, ($p=0.05$; Figure 7 and Online Video II)

Similar magnitude of change of the “scar-injected and non-injected” with a transmural extent of $>50\%$ and $<50\%$

“Scar-injected” and “Scar non-injected” were grouped based on baseline transmural extent of $>50\%$ and $<50\%$. For the “scar-injected”, there was a similar magnitude of change in SEED and SEF regardless of the transmural extent (between group comparison $p>0.05$ for all analyses): SEED decreased for the “scar-injected” segments with a transmural extent of $>50\%$ (from $9.8\pm 1.3\text{g}$ to 5.2 ± 0.7 , $n=25$, $p<0.0001$), and transmural extent of $<50\%$ (from 10.4 ± 3.3 to 6.7 ± 5.8 , $n=5$, $p=0.03$). For SEF, the “scar-injected” segments SEF with a transmural extent of $>50\%$ increased from $21.8\pm 3.8\%$ to $28.8\pm 4.0\%$, $n=25$, $p=0.008$), and showed a trend of improvement in segments with a transmural extent of $<50\%$ (from 11.0 ± 2.6 to 15.1 ± 2.9 , $n=5$, $p=0.09$; Figure 7).

Transmural infarct size reduces also in “scar non-injected” segments

For “scar non-injected” in patients treated with MSCs, we observed that the scar with a baseline transmural extent of >50% had a similar magnitude of change in SEED with the “scar-injected” segments. SEED decreased from 11.6 ± 1.5 to 8.6 ± 1.1 , $n=29$, $p=0.001$. For SEF, the “scar non-injected” with a transmural extent of >50% did not show any change (from 21.3 ± 2.8 to 23.8 ± 3.5 , $n=29$, $p>0.05$) (Figure 7).

DISCUSSION

The current study has three new major findings: First, the actual site of injection of MSCs plays a fundamental role in the response to cell therapy for chronic ischemic heart disease. Injected sites respond with a reduction in scar tissue and improvement in wall thickening accompanied by functional recovery. Second, while remote segments also exhibit reduction in scar, these segments do not exhibit comparable improvement in myocardial contractility. Finally, non-scarred segments do not improve functional contraction even when cell injections are delivered. These three major findings were similar when considering transmural vs. non transmural injected scarred segments. Together these findings offer both mechanistic and practical insights regarding this new and potentially important form of therapy for chronic heart disease.

These findings can best be viewed in the context of mechanistic findings from preclinical models^{2, 10, 11, 21} and clinical trials^{3, 4, 22-25} and are in agreement with both the direct and indirect mechanisms that underly these MSC actions²⁶. Directly, MSCs are known to engraft and differentiate preferentially in the infarct / border zone^{2, 10, 11} producing both reduction of scar size and contractile restoration^{2, 11, 27}. In addition, MSCs can release paracrine factors^{8, 12, 28} which can have local and remote effects; for example the release of matrix metalloproteases^{8, 12, 28} can contribute to scar size reduction, an effect that could occur remotely. Thus, the improvement in ventricular architecture is driven by a global reduction in scar size which is accompanied by improved contraction that occur preferentially in the injected myocardial segments. These data show that the site of injection matters and that MSCs generate scar size reduction and functional restoration regardless of the transmural extent.

The direct and indirect mechanisms underlying these MSC actions have been previously explored in a robust and representative preclinical swine model of ischemic cardiomyopathy^{29, 30}. Using this model, our group and others^{2, 10, 31} demonstrated that MSCs engraft into chronically scarred myocardium¹¹, undergo trilineage differentiation^{10, 11}, promote neovascularization^{21, 31, 32}, and enhance the proliferation and differentiation of endogenous cardiac stem cells³³ in the infarct territory,^{10, 21} especially at the interface between scar tissue and bordering viable myocardium^{2, 10, 11}. As a result MSCs lead to reduction of scar size, and to improvement in LVEF³⁴. Moreover, direct hemodynamic assessments of contractility, lusitropy, and myocardial energetics have shown global ventricular improvement including preload recruitable stroke work (PRSW), an integrated measure of hemodynamic studies²⁷.

Another clinical study of intramyocardial MSC therapy that used cardiac magnetic resonance (CMR) analysis, also demonstrated a relationship between regional restoration of

myocardial contractility, assessed as peak Eulerian circumferential strain (Ecc)⁸, and reverse ventricular remodeling. In that study, improved Ecc in the scar border zone occurred at 3-months after cell injection and correlated with a reduction in ventricular volumes at 12-months after cell therapy. The results of the Cardiopoietic stem Cell Therapy in heart failure (C-CURE) study also showed that MSCs targeted to viable but dysfunctional LV segments resulted in reduction in scar size and increased LVEF in chronic ischemic cardiomyopathy patients treated with autologous cardiopoietic MSCs²².

The imaging approach used in the POSEIDON study provided the means to test the differential effects of local vs. remote (paracrine) effects on function and scar size. In the POSEIDON trial not all scarred myocardial segments were injected allowing a comparison of TESI-treated and non treated myocardial segments. Indeed, we found a divergent response regarding functional restoration and scar reduction. While myocardial scar segments treated with TESI showed a greater reduction in scar size than those not treated with TESI, the latter also had a significant reduction in scar size. However, only TESI-treated myocardial scar segments exhibited a significant improvement in SEF, indicating that direct cell effects preferentially augment functional restoration. Furthermore, treated non-scarred myocardial segments did not show changes in contractility, deriving that the changes happening in the infarcted myocardium favor the mechanisms underlying MSCs therapy. These data also demonstrate that there is a radius of activity for these phenotypic responses: MSCs lead to scar size reduction and restoration in SEF also in “adjacent” myocardial segments (around “scar-injected” sites), while remote segments only showed reduction in scar size. Collectively, these findings demonstrate a dichotomy in the mechanisms driving the functional (local) and reverse remodeling (local and remote) responses to MSC therapy. Clinically, this suggests that new therapeutic strategies for TESI should be anatomically personalized, with an individualized patient-specific approach based not only on location and infarct size but also number of injections, dose, and concentration of cells.

In the POSEIDON study⁴, a paradoxical response to the cell dose was found. When comparing 20 vs. 100 vs. 200 million MSCs, the greatest benefit in total scar size reduction was seen in the group that received 20 million cells. Similar observation was seen in the current results, with a greater reduction of scar size in the scar-injected segments receiving 20M of MSCs. Among all the groups, 20M of autologous MSCs showed a greater reduction of scar size which is consistent. Variables which may affect the response to treatment thus include cell number (total dose), cell concentration and the number and distribution of injections. The small sample size of our study limits the possibility of making definitive conclusions but brings the basis for better strategies in stem cell therapy. Optimization of the response to TESI is likely to include a number and pattern of injections specific to a given infarction. In this segmental analysis, a linear positive correlation was noted between the number of myocardial segments with scar treated by TESI and the improvement of SEF. A higher number of myocardial segments with scar treated by TESI could promote a greater improvement; nonetheless, other factors also need to be explored. In the SCIPIO clinical trial^{23, 35}, patients received cardiac stem cells by intracoronary infusion; both the infusion site and the cell dose were customized according to the infarct location and size. Patients who were followed up with CMR were analyzed for regional EF. The SEF showed

improvement to 24.5% at 4-months and to 28.2% at 12-months in the infarct-related segments. This improvement was even greater when the dysfunction was severe. Similarly, we observed improvement in contractility in the infarcted segments, and that the lower the SEF at baseline the greater the improvement at 13-months after MSC therapy. In light of the above, we can argue that individualized tailoring including the number of stem cell injection sites and the total cell dose may offer an optimal strategy compared with other fixed approaches which do not account for different locations and extent and severity of the infarction.

Together these findings support the biological activity of mesenchymal stem cell therapy to reverse pathologic remodeling through a process driven by infarct size reduction. These changes are associated with improvement in myocardial contraction that is preferentially found in the scar tissue zones. These data suggests the duality of the functional benefits of MSCs, involving both direct local and also diffusible, potentially paracrine mechanisms. What remains to be determined is whether the local effect of the injected MSCs is specific for structural and/or functional repair and whether it is more effective or even more enduring than the paracrine effects. Moreover, it is unknown whether the local cellular and paracrine effects happen in synchrony or even in synergy.

Conclusions

The study of segmental myocardial dynamics can expand the current understanding of local changes that guide cardiac remodeling following TESI. This study revealed that significant improvement in SEF occurred in myocardial scar segments treated with TESI but not in myocardial scar segments without TESI, whereas scar size reduction was evident in TESI treated and non-treated segments. We also demonstrated that myocardial segments with severe regional dysfunction, SEF<20%, showed even more significant improvement in SEF, suggesting that more impaired myocardial segments have better response to TESI. Collectively, these findings suggest that local and paracrine factors differentially influence functional restoration and reverse remodeling. Although this study does not answer the mechanistic issues underlying the functional and structural responses to MSC therapy, it lends support to the transformation of cell therapy into a more customized, targeted therapy. Myocardial regional assessment tools, such as SEF and myocardial SEED, should be considered as potential measures of efficacy for cardiac stem cell therapy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Therapy Data and Safety Monitoring Board (DSMB). Biocardia Inc provided the Helical Infusion Catheters for the conduct of POSEIDON.

Nonstandard Abbreviations and Acronyms:

TESI	Transendocardial Stem Cell Injection
EED	Early Enhancement Defect
SEED	Segmental Early Enhancement Defect
SEF	Segmental Ejection Fraction
LAO	Left anterior oblique
RAO	Right anterior oblique
EF	Ejection Fraction
POSEIDON	Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis
MSC	Mesenchymal Stem Cell
LV	Left ventricular
MDCT	Multidetector Computed Tomography
AHA	American Heart Association
HU	Hounsfield units
LAD	Left anterior descending
PRSW	Preload Recrutable Stroke Work
Ecc	Eulerian circumferential strain
CMR	Cardiac Magnetic resonance
DE	Delayed enhancement
C-CURE	Cardiopoietic stem cell therapy in heart failure

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Novelty and Significance

What Is Known?

- Transendocardial Stem Cell Injection (TESI) with mesenchymal stem cells (MSCs) improves remodeling, decrease infarct size, and improves function and quality of life in chronic ischemic heart disease.
- MSCs mechanisms include anti-fibrotic effects, neovascularization, neomyogenesis and stimulation of endogenous cardiac cells.
- Whether these actions are exerted locally, at a distance, or globally, and the impact of precisely localizing cell injections, remain unclear.

What New Information Does This Article Contribute?

- The injection site for cardiac cell therapy has an important impact on the phenotypic response, such that cardiac function improved preferentially at injection sites whereas scar reduction was evident both locally and remotely to sites of cell delivery.
- Cell injections enhanced ventricular performance to a greater extent at sites that had greater baseline dysfunction.
- Cell therapy reduced fibrosis in infarct scars that were both transmural and non-transmural.

We performed a regional analysis after TESI, to test the hypothesis that sites of cell injection respond more favorably in terms of cardiac repair than sites not receiving cell injections. We report three new major findings. First, injected scarred areas respond with a reduction in fibrotic tissue and improvement in wall motion. Second, while segments remote to the injected area also exhibit reduction in scar, they do not exhibit comparable improvement in performance. Finally, non-scarred segments do not improve performance with TESI. These findings elucidate a dichotomy in the phenotypic response to MSC injections, demonstrating that local and paracrine factors differentially influence functional restoration and scar size reduction. Locally, there is evidence of reduction of scar size associated with significant improvement in performance, whereas remotely only scar size reduction is seen. Collectively, these findings can guide a personalized approach for optimizing the response to cell therapy in humans by tailoring the injection strategy to patient anatomy.

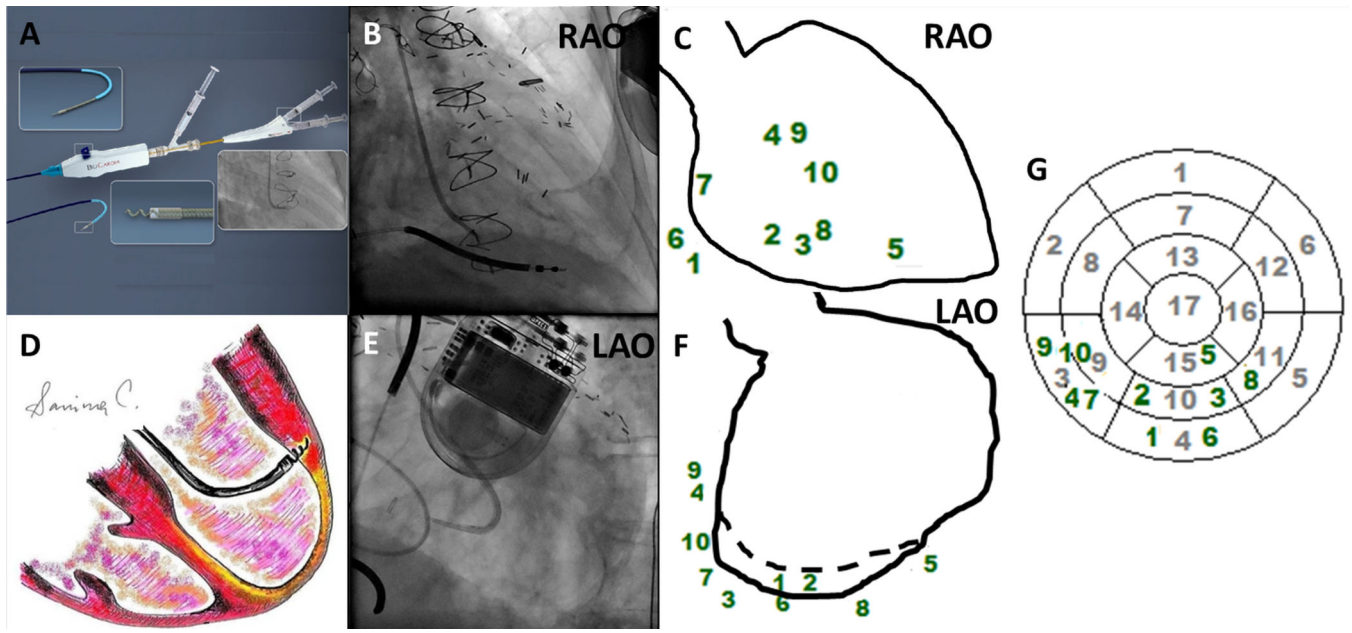


Figure 1. Allocation of 10 injections using biplane ventriculography during Transendocardial Stem Cell Injection

(Panels A and D) Biocardia helical infusion catheter and schematic depiction of an intraendocardial injection, respectively. (Panels B and E) Biplane fluoroscopy was used for navigation. (Panels C and F) Every injection site was marked in two projections using ventriculographic diastolic contours and translated onto a 17-segment polar map (Panel G) to identify all segments that received intramyocardial injections of MSCs.

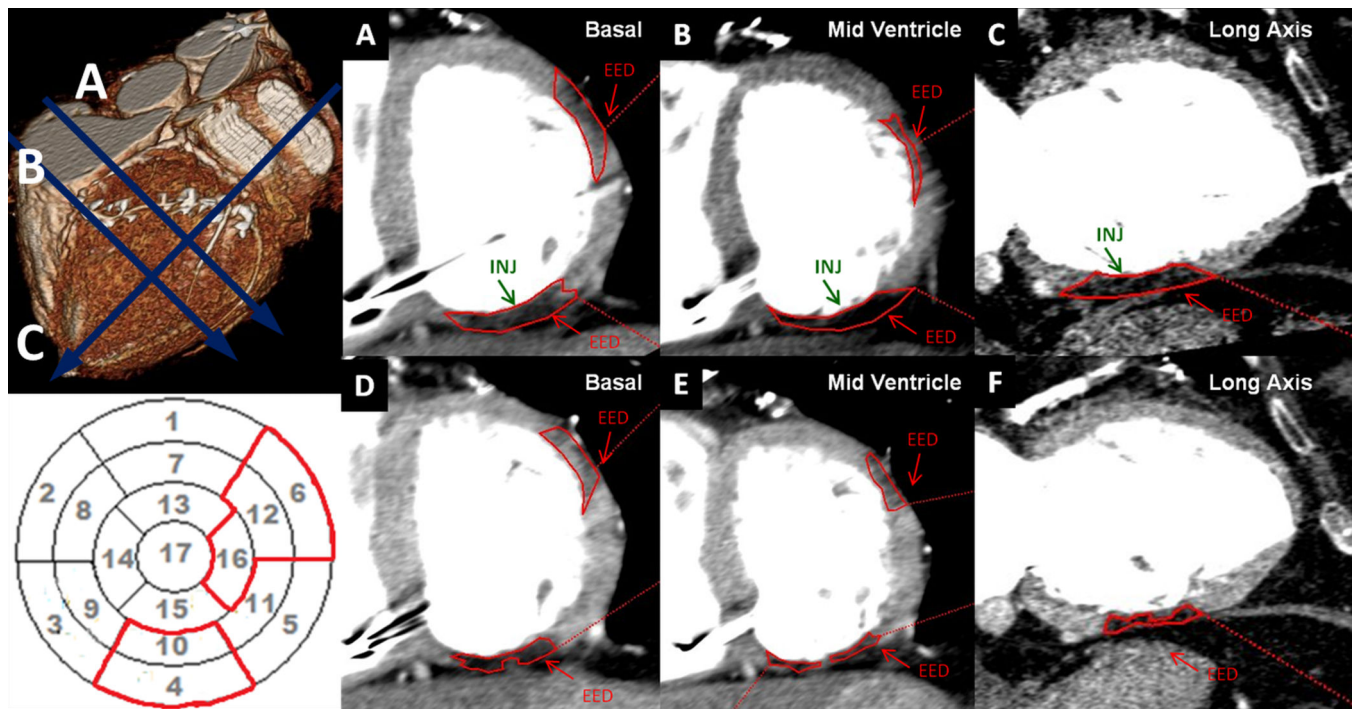


Figure 2. Greater reduction in scar injected segments

(Upper left Panel) 3D reconstruction at baseline and MDCT images with 20HU below the normal myocardium density at (Panel A) basal short-axis, (Panel B) mid-ventricle short-axis and (Panel C) long-axis representing EED areas delineated in red. (Lower left Panel) Polar map reconstruction with EED delineated in red. Scar mass (EED) at baseline (Panels A-C) has a greater reduction in the injected segments (marked by INJ) located in inferior segments at basal, mid-ventricle and long-axis, from 5.9g to 1.4g compared to (Panels D-F) non-injected lateral segments at basal, mid-ventricle and long-axis images, from 4.6g to 1.7g thirteen months after MSCs.

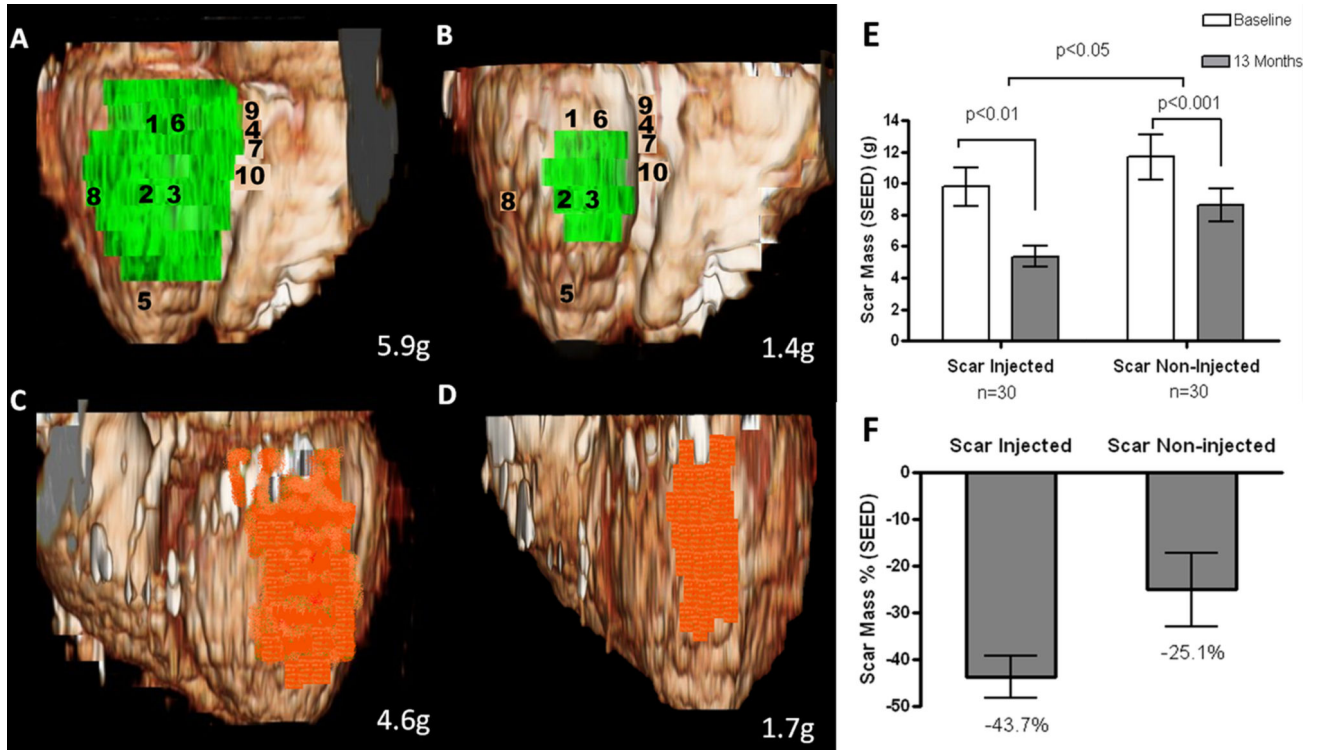


Figure 3. Volume rendered 3D reformats of left ventricle with color encoding of scar tissue (Panels A and B) Scar mass (green) of inferior segments treated by TESI at baseline and 13-months post TESI, respectively (numbers represent sites of injection). (Panels C and D) Scar mass (orange) of lateral segments not treated by TESI at baseline and at 13-month follow up, respectively. Actual scar mass (grams) is depicted in the lower right corner of each panel. 3D-reconstructions in this figure correspond to SEED measurements of the same patient as in Figure 2 and Online Video I. (Panels E and F) Absolute values and %changes of scar mass obtained by segmental imaging analysis approach. When considering the autologous and allogeneic groups combined, there is greater scar size reduction in the “scar-injected” segments ($-43.7 \pm 4.4\%$, from 9.8 ± 1.2 to 5.4 ± 0.7 g, $n=30$, $p<0.01$), compared to the “scar-non-injected” segments ($-25.1 \pm 7.8\%$, from 11.7 ± 1.4 to 8.6 ± 1.0 g, $n=30$, $p<0.001$; between group comparison “scar-injected” vs. “scar-non-injected” $p<0.05$).

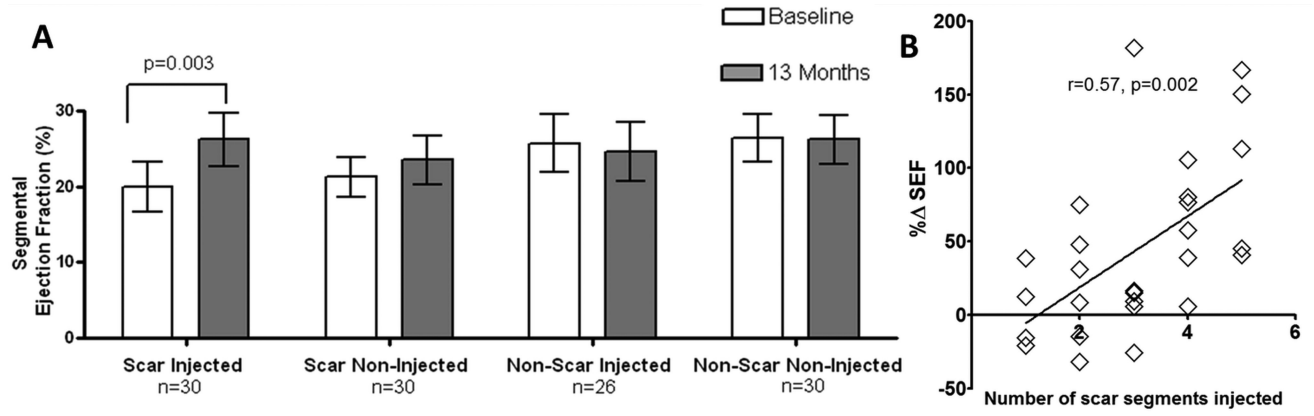


Figure 4. Restoration in contractility (SEF) and association between number of injected segments vs. SEF

(Panel A) Greater improvement in SEF was observed in patients with more scar (SEED) injected segments, when considering the autologous and allogeneic groups combined, “scar-injected” segments SEF improved from 19.9±3.3% to 26.3±3.5% at 13-months after TESI (p=0.003). Conversely, SEF did not significantly improve when “scar-non-injected” segments were evaluated. The “non-scar-injected” and “non-scar-non-injected” segments did not demonstrate changes in SEF at 13-months. (Panel B) Pearson correlation coefficient $r=0.57$, 95% CI, 0.23 to 0.78; two-tailed p value=0.002.

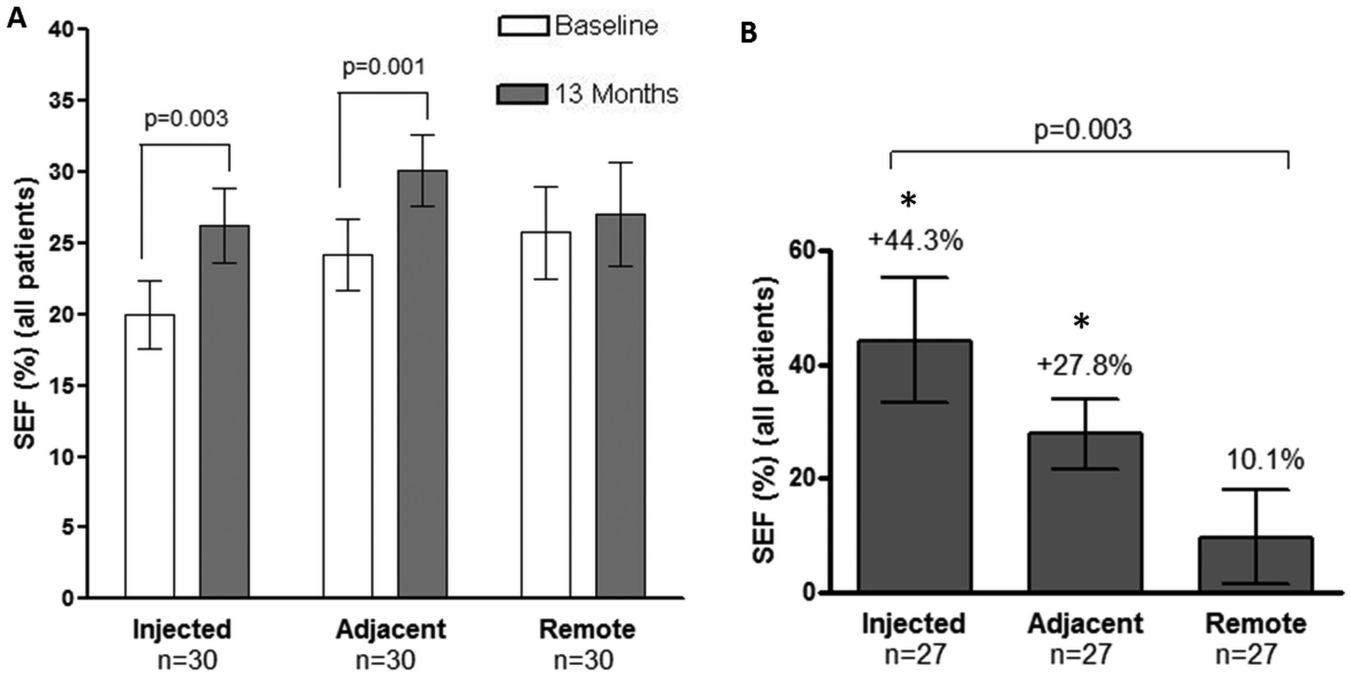


Figure 5. Radius of Activity for Transendocardial Stem Cell Injections (TESI) with Mesenchymal Stem Cells in Segmental Ejection Fraction (SEF%)

(Panel A) Improvement in contractility was observed in “scar-injected” (from 19.9±3.3% at screening to 26.3±3.5% at 13-month after TESI (p=0.003), and “adjacent” segments (from 24.2±2.6% to 30.1±2.6%, n=30, p=0.001). Conversely, the “remote” segments did not have changes in contractility (15.8 ±12.4% from 25.8±3.3% to 27.1±3.7%, n=30, p=0.4); between group comparison p>0.05). (Panel B) Percent changes from baseline in SEF showed changes by +44.3%±11.2% in “scar-injected”, by +27.8±6.4% in “adjacent” and by 10.1±8.9% in the “remote” segments (one way analysis of variance p=0.003. Dunn's posttest *p<0.05 in “scar-injected” vs. “remote” and “adjacent” vs. “remote”).

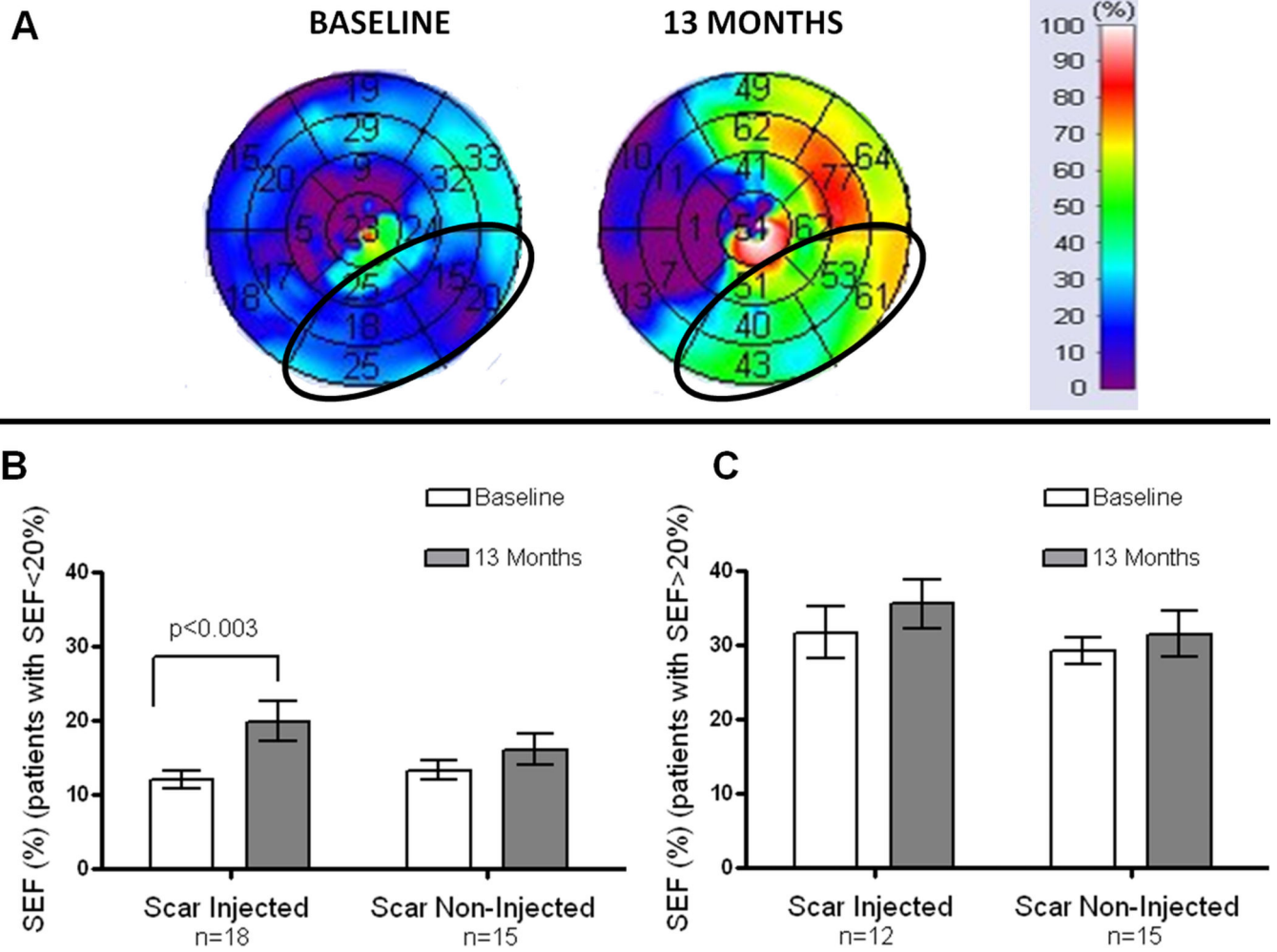


Figure 6. Improvement in contractility is particularly evident in the highly dysfunctional segments

(Panel A) SEF<20%, scar-injected segments (baseline) which are encircled with an ellipse in the 17-segment polar map with their corresponding improvement after injection of mesenchymal stem cells. (Panel B) Represents the changes in SEF with highly dysfunctional segments (SEF<20%), the “scar- injected” SEF was 12.1±1.23% at baseline and improved to 19.9±2.69% (p=0.003) at 13-months after TESI (n=18 patients). In this subgroup analysis, the “scar-non-injected” SEF (n=15 patients) showed a trend of increase from 13.3±1.3% to 16.1±2.13%, (p=0.05).(Panel C) Shows changes in segments with SEF>20%.

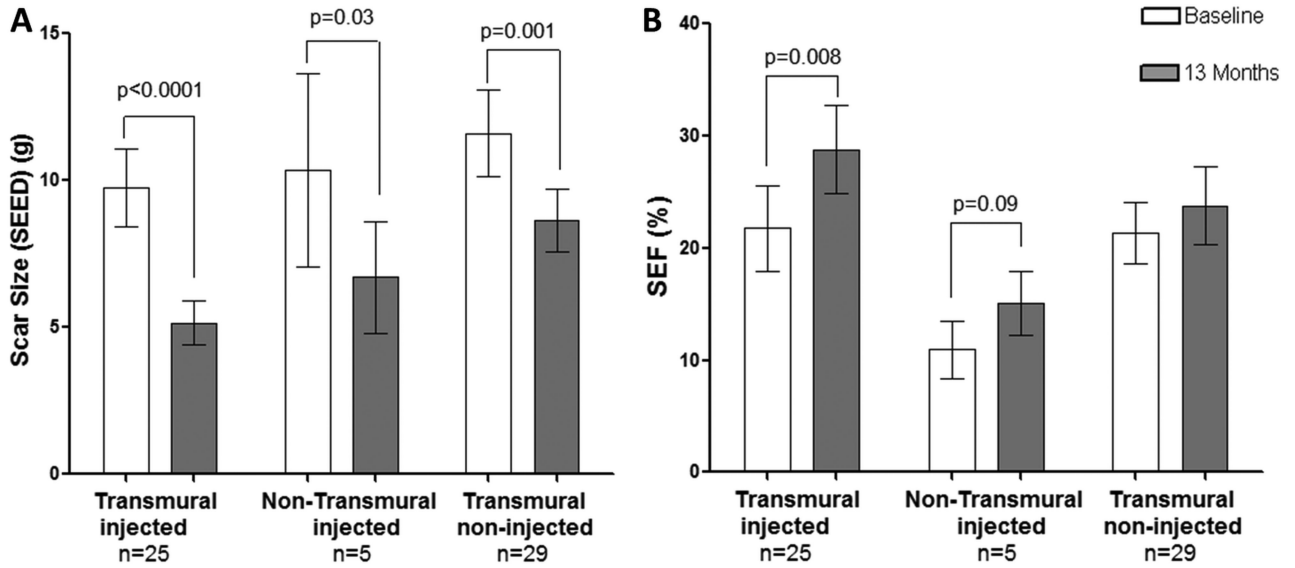


Figure 7. Dual effects of MSCs, global scar size reduction but restoration of contractility only in scar-injected segments, regardless of transmurality

(Panel A) Similar magnitude of change in SEED of the “scar-injected” and “non-injected” with a transmural extent of >50% (from 9.8±1.3 to 5.2±0.7g, n=25, p<0.0001), and <50% (from 10.4±3.3 to 6.7±5.8g, n= 5, p=0.03). (Panel B) Similar magnitude of change in SEF only in the “scar-injected” with a transmural extent of >50% (from 21.8±3.8% to 28.8±4.0%, n=25, p=0.008) and <50% (from 11.0±2.6 to 15.1±2.9, n=5, p=0.09).