HOSPITAL CHRONICLES 2008, 3(3): 77–85

REVIEW

Stem Cells and Cardiac Repair

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ABSTRACT

Contrary to the initial belief that the heart is a terminally differentiated organ that cannot replace its own cell damage, there is now proof that the circulating blood provides the injured tissue with adult stem and progenitor cells, which have the potential to differentiate into cardiomyocytes and ultimately improve cardiac function. Thus, transplantation of stem cells into the myocardium in patients with severe myocardial dysfunction post-myocardial infarction is being currently investigated for experimental as well as for clinical purposes. Many issues regarding the mode of action remain to be elucidated. The BOOST trial was the first completed, randomized study that showed safety, feasibility and efficacy of the method. However, a more recent double-blind, placebo-controlled study failed to reveal an increase in global left ventricular ejection fraction and cast doubt on the efficacy of the method. Thus, further randomized studies are needed to evaluate this novel approach in the treatment of ischemic heart failure and determine its role, safety and efficacy.

KEY WORDS: *stem cells; myocardial regeneration; myocardial infarction; skeletal myoblasts; cardiac repair*

Until recently it was believed that the heart is a terminally differentiated organ with no ability to repair any tissue injury. However, recent evidence suggests[1-4] that the myocardium may have some potential to repair its own tissue injury, but this regenerative ability is limited and can only repair minor cardiomyocyte damage. Any tissue loss that outbalances the regenerative and self-renewing properties of the heart would lead to an overt clinical picture of heart failure.

The healing process of the myocardium is undertaken by cardiac stem cells, the origin of which is a subject of debate. These cells might represent either local resident cardiac stem cells[3-9] that have been suggested to differentiate into cardiomyocytes or bone marrow derived stem cells that reside in the myocardium as part of the natural myocardium homeostasis mechanism or in response to tissue damage[10-12]. In a recent report by Laugwitz and colleagues[7], an endogenous cardiac progenitor (cardioblast) was identified in the postnatal rat, mouse and human myocardium. This cell is capable of obtaining a mature cardiomyocyte phenotype, expressing myocytic markers and generating action potentials. The epicardium has also been suggested as a potential source of cardiac stem cells, which then migrate into the myocardium and transdifferentiate into multiple cell lines[13].

Cardiac repair is a very complex and not fully understood mechanism that comprises many interacting steps. Certain cytokines play a critical role in this cascade,

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Presented in part at **"Cardiology Update 2006"**, International Cardiology Symposium of Evagelismos General Hospital of Athens, Athens, Greece, April 13-15, 2006

that act as mediators of this complex process. Damaged tissue secretes stem cell factor (SCF), CXCR4, stromal-derived factor-1 (SDF-1), granulocyte colony stimulating factor (G-CSF), vascular endothelial growth factor (VEGF), monocyte chemoattractant proteins-1 (MCP-1) and other substances, which determine the mobilization and recruitment of stem/progenitor cells to the injury site in the periphery. Homing of the activated stem cells to the injury site is the next crucial step as they adhere to the activated endothelium, migrate through the endothelial cells and finally invade and home in the damaged myocardium. The final step of this cascade is the transdifferentiation of the integrated stem cells, which is a process determined not only by their genetic material but also but the microenviroment they are attracted to[14-16].

With all experimental data showing improvement of cardiac function after stem cell and skeletal myoblast transplantation, regardless of the exact mode of action, the next logical step was the conduction of studies in humans. Most of them were small, uncontrolled and nonrandomized, thus causing difficulties in estimating the exact role of cardiac repair therapy in patients with acute or chronic coronary artery disease[10,11,17-20].

A. PATIENTS WITH NON-VIABLE, CHRONICALLY ISCHEMIC CARDIOMYOPATHY

In patients with chronic ischemic cardiomyopathy as a result of postinfarction scars, Pagani and colleagues injected autologous skeletal myoblasts intramyocardially in 5 patients awaiting heart transplantation during left ventricular assist device (LVAD) implantation. Examination of the hearts from 3 patients who finally underwent transplantation and from one who succumbed revealed development of myotubes in the scarred myocardium.21 Menasche and colleagues implanted autologous skeletal myoblasts intramyocardially (in-scar injection) in 10 patients with severely depressed left ventricular (LV) function (LV ejection fraction-EF <35%) during coronary artery bypass grafting (CABG) surgery. Bypass grafting was carried out in noninjected territories only. Almost one year later he reported an improvement in regional wall motion and global LVEF, but 4 patients had to undergo cardioverter-defibrillator implantation due to episodes of sustained ventricular tachycardia[22].

Almost similar in design was the study by Siminiak and colleagues[23], who also combined CABG surgery, with grafting both injected and noninjected territories, in 10 patients with severely compromised LV function and intramyocardial skeletal myoblast injection in the akinetic/dyskinetic areas. Four months later there was an improvement in regional and global contractility, which was sustained over a period of 12 months. Ventricular arrhythmias, especially in the early postoperative stage, were also a serious concern in this group of patients.

Autologous skeletal myoblasts were also injected intramyocardially by 2 other groups and were also combined with surgical revascularization treatment. Herreros and colleagues[24] treated 11 patients while revascularizing both injected and noninjected segments, whereas Chachques and colleagues[25] treated 20 patients in combination with revascularization of the noninjected territories only. Both groups reported an improvement in regional and global contractility as well as enhancement of viability in the injected area. An interesting finding in the former study was the lack of cardiac arrhythmias and especially ventricular tachyarrhythmias in contrast to the 2 aforementioned studies by Pagani and Menasche. This finding is probably associated with the selection of patients with higher baseline LVEF and to the effect of revascularisation on the infarcted myocardial segments during CABG surgery.

The percutaneous, transendocardial route reported by Smits[26] consisted of targeted intramyocardial injection of autologous skeletal myoblasts guided by electromechanical mapping. This group treated 5 patients with heart failure and after a follow-up period of 6 months, cell transplantation resulted in an increase of LVEF and improvement of wall thickening at areas of injection. This technique was also complicated by serious ventricular arrhythmias or even reported sudden deaths, that made the implantation of cardioverter-defibrillator (ICD) mandatory. Siminiak and colleagues[27] applied in a phase I trial a novel technique of trans-coronary-venous for skeletal myoblast transplantation (TransAccess catheter system) in 9 patients with akinetic/dyskinetic scar tissue and moderately depressed LVEF. The process was feasible and safe, but he only reported an improvement of New York Heart Association (NYHA) functional class and a nonsignificant LVEF increase in a period of 6 months.

A recent study of a 4-year follow-up[28] in 30 patients who underwent in-scar injection of skeletal myoblasts during CABG surgery (n=24) or LVAD implantation (n=6) reported new evidence of cell viability in the region of myocardial scar, an increase in LVEF (from 28% at baseline to 36% at 2 years) and a reduction in LV dimensions. However, these results on cardiac function should be interpreted with caution since skeletal myoblast transplantation was associated with bypass surgery. Histological analysis of 4 of 6 patients from the LVAD arm who underwent heart transplantation revealed survival of skeletal myoblasts in the infarcted myocardium, minimal localized migration of myoblasts and myofiber formation. The procedure was safe, with absence of increased arrhythmogenicity and deaths in the follow-up period that could be attributed to cell therapy. The only procedure-related arrhythmia was nonsustained ventricular tachycardia in 3 of 24 CABG patients and was managed with medications and ICD implantation.

Only a few groups used bone marrow derived cells for transplantation in this cohort of patients with chronic

ischemic heart failure and postinfarction scars. Stamm and colleagues[29,30] injected selected CD133+ bone marrow stem cells along the infarct border zone during CABG surgery in patients with depressed LVEF. These studies were safe and resulted in improvement of perfusion in the cell-treated area, global LVEF and favorable LV remodeling.

Strauer and colleagues reported recently the results of the IACT study, which showed a favorable effect of intracoronarilly (in the infarct-related artery) infused unselected bone marrow derived mononuclear cells on functional and metabolic regeneration of chronic, non-viable myocardial infarction (MI). This study included also a nonrandomized control group. Cell therapy which was applied in 18 patients resulted in an improvement of global and regional LV function, reduction of infarct size and enhanced regional viability of the infarcted tissue 3 months later. There was no evidence of arrhythmia exacerbation and only one patient (out of the 18 treated) showed significant restenosis which was successfully treated with stent implantation[31].

Thus, in the clinical setting of nonviable, postinfarction scarred myocardium, most studies conducted are small, nonrandomized and used intramyocardial injection of skeletal myoblasts, with surgical or percutaneous approach. Most of them showed increase in LVEF and regional contractility but the effects of the cell treatment should be assessed cautiously due to the concomitant revascularization procedure. Increased arrhythmogenicity, including ventricular dysrrhythmias and deaths also raises concern about the potential for widespread use. Recent work presenting use of bone marrow-derived selected or unselected stem cells in the same clinical setting also seem efficacious without any serious side effects.

B. PATIENTS WITH CHRONICALLY ISCHEMIC BUT VIABLE MYOCARDIUM

Hamano and colleagues[32], for the first time, injected intramyocardialy autologous unselected bone marrow-derived mononuclear cells during CABG in 5 patients with old MI and chronic, severe ischemic cardiomyopathy. After 12 months, they reported enhancement of myocardial perfusion and the safety of the method. In the next 2 studies published in 2003, autologous unselected bone marrow stem cells (BMSCs) were injected transendocardially (guided by electromechanical mapping) in small, uncontrolled studies. At 3 months, all patients experienced less angina attacks and enhanced myocardial perfusion[33,34], while magnetic resonance imaging (MRI) also revealed improved regional wall motion in the injected areas[33]. In the only study with a nonrandomized control group[35]. Perin and colleagues also used the percutaneous transendocardial (electromechanically guided) approach in 14 patients with severe, chronic ischemic heart failure with a mean LVEF of 30%. Two months later, they reported less angina attacks, improved NYHA functional class, enhancement of perfusion, regional wall motion, global LVEF and a significant reduction in left ventricular end-systolic volume in the cell treated group. One patient died 3 months after cell treatment. A recent report from this group[36] focuses on the postmortem analysis of the heart of one patient who received cell therapy. In the cell treated myocardium, enhanced angiogenesis was present, partially combined with a special pericyte population, which was located in areas suggesting migration towards adjacent bundles of cardiomyocytes. These pericytes were characterized not only by the presence of cytoskeletal elements and contractile proteins (troponin, sarcomeric α-actinin, actinin) but also by the expression of specific myocardial proteins, suggesting a partial transdifferentiation.

A recent randomized, double-blind placebo-controlled study[37] assessed the impact of intracoronarilly delivered blood-derived circulating progenitor cells (mobilized with G-CSF) combined with recanalization of chronic coronary total occlusion on hibernating myocardium. At 3 months, coronary flow reserve in the reperfused artery improved, the number of hibernating myocardial segments treated declined, the infarct size was reduced and LVEF increased significantly in the treatment group. Of note that no serious adverse events were reported including in-stent reocclusion or in-stent restenosis.

Thus, in the setting of chronic, severe hibernating and/or ischemic cardiomyopathy, transendocardial (electromechanically guided) approach seems to increase myocardial perfusion and probably regional wall motion, but only two studies[35,37] showed an increase in global LVEF. All studies reported substantial relief from angina pectoris, but this could be attributed to a significant placebo effect. This technique seems to be feasible and safe, but final conclusions cannot be drawn from these small, uncontrolled studies.

C. PATIENTS IN THE EARLY POSTINFARCTION PERIOD

The most widely investigated clinical scenario however concerns patients during the early postinfarction period, who undergo percutaneous coronary intervention (PCI) and stent implantation of the infarct related artery (IRA), through which several days later selected or unselected BMSCs are infused. Strauer and colleagues[38], delivered in 10 patients intracoronarilly-via the central lumen of an over-the-wire system- unselected BMSCs, 5-9 days post MI, whereas 10 other patients served as a nonrandomized control group. Three months later they reported a decrease in the infarct size and an increase in myocardial perfusion and improvement in regional wall motion, while global LVEF and LV dimensions remained unchanged.

Another group that used unselected bone marrow stem

cells and a nonrandomized control group was that of Fernandez-Aviles and colleagues. They also delivered (in 20 patients) mononuclear cells intracoronarily almost 2 weeks after MI. At 6 months, they reported an improvement in regional wall motion, global LVEF, LV endsystolic volume and increased thickness of the infarcted wall[39].

Assmuss and colleagues[40] administered intracoronarily BMSCs almost 4 days after MI and subsequent PCI with stent implantation. Eleven patients received circulating blood-derived progenitor cells (CPCs), 9 patients received bone marrow-derived mononuclear cells (BMCs) and 11 patients served as a nonrandomized, control group. At 4 months follow-up, they reported a significant increase in global LVEF, regional wall motion in the infarcted territory, coronary flow reserve of the infarct related artery, the viability of the infarcted segment and also improvement of the LV end-systolic volumes in both cell treated groups compared to the control group. Of note that no malignant arrhythmias in either cell-treated group were observed and that there was no difference in the above mentioned parameters between the two different cell groups administered.

In the context of the same study, Schachinger and colleagues[41] also reported a sustained improvement in LV remodeling and cardiac function in patients randomized to intracoronary infusion of either circulating blood-derived progenitor cells (30 patients) or bone marrow-derived mononuclear cells (29 patients) as a complementary therapy to stenting of the IRA. At 4 months, quantitative LV angiography revealed a significant increase in LVEF and reduction in LV end-systolic volumes with no difference between the 2 cell groups. Cardiac performance was also assessed by MRI at 4 and 12 months after cell treatment. Interestingly, global LVEF not only improved significantly in the first 4 months, but increased further in the next 8 months, thus resulting in a total increase of almost 9% one year after cell therapy.

Another group administered selected BMSCs, namely MSCs, via the intracoronary route in the infarct related artery 18 days after MI and subsequent PCI[42]. Thirty-four patients were randomized for cell therapy (4.8-6x10^{10} cells) and 35 for the control group. At 3-6 months follow-up, the cell-treated group showed a decrease in the size of the infarction, LV dimensions and an improvement in regional and global contractility. Even though intracoronary delivery of MSCs in dogs[43] was associated with "microinfarctions" due to the size of this particular cell type, this study did not report any periprocedural adverse events. Autologous culture-expanded MSCs combined with EPCs were administered intracononarilly also by another group in patients with both recent and old anteroseptal MIs[44]. Four months after cell therapy, they reported an uneventful course and partial improvement in contractility and viability in some of previously non-viable myocardial segments, but no effect on global LVEF or LV remodeling.

Intracoronary infusion of selected BMSCs in patients with acute MI and PCI with stenting of the IRA was also reported by Bartunek and colleagues[45] who administered 12.6±2.2x10^6 bone marrow-derived CD133 positive progenitor cells in 19 patients 12 days after acute MI while 16 patients served as a nonrandomized control group. At 4 months, there was a significant improvement in myocardial perfusion, viability and function and absence of stem-cell related arrhythmias in the cell-treated group. On the other hand, the cell treated-group showed in-stent restenosis (7/19 patients) and reocclusion (2/19 patients) as well as significant de novo lesion of the IRA (2/19 patients).

In the BOne marrOw transfer to enhance ST-elevation infarct regeneration (BOOST) trial, 30 patients received 2.5±0.9x10^9 unselected bone marrow-derived nucleated cells intracoronarily and 30 patients served as the control group Approximately 6 days after successful PCI and stent implantation in the IRA[46]. At 6 months, cardiac MRI revealed significant improvement of global LVEF in the cell-treated group due to an increased systolic wall motion of the segments adjacent to the infarcted segment. Cell therapy did not increase in-stent restenosis, arrhythmogenic complications or cause microinfarctions. There was no change in LV dimensions or viability of the infarcted area. Based on this latter finding, Wollert and colleagues attributed the recovery of the cardiac function probably to the paracrine effects of bone marrow-derived stem cells, and not to their transdifferentiation into cardiomyocytes.

Very recently the benefit of bone marrow-derived unselected stem/progenitor cell therapy was questioned in a truly randomized, double-blind, placebo-controlled study in 67 patients after PCI for ST-elevation MI[47]. At 4 months, no additive effect of cell therapy was noted on global LV functional recovery in patients with moderately depressed global LV function. Whether this novel treatment modality could be of practical use in patients with greater myocardial area at risk and greater LV dysfunction still has to be elucidated with similarly designed trials.

Thus, in the setting of the early postinfarction period clinical trials use mostly unselected bone marrow stem cells via the intracoronary over-the wire approach in the IRA. They show improvement of regional wall motion contractility of the infarcted or the infarct border area, which accounts for the enhancement of global LVEF reported in most of them. On the other hand, the fact that there are confounding data on the actual effect of BMSCs on myocardial perfusion, infarct size and LV dimensions, emphasizes the need to further elucidate the mechanisms of action of these cells. One important advantage of this cell type is the lack of adverse events and especially ventricular arrhythmias or sudden cardiac deaths.

TABLE 1. Clinical Studies Using Cytokines for Stem Cell Mobilization

Study	Clinical setting	Patients treated	Cell type infused	Delivery route	Outcome/Adverse events
Kang et al [62]	Onset of G-CSF treatment 4 days before PCI for MI (MI 2-270 days old)	G-CSF only (10 pts) vs apheresis after G-CSF (10 pts) vs controls	Peripheral collected MNCs (mobilized with G-CSF)	ic	Improvement in systolic function and myocardial perfusion (ic cell infusion). Mild elevation of CPK-MB (ic cell infusion group). Increased rate of in-stent restenosis of the IRA (Only G-CSF group).
Ince et al [63]	Onset of G-CSF treatment 89±35 minutes after primary PCI	PCI + G-CSF (25 pts) vs PCI (25 pts)	NA	NA	Improvement in systolic function, LV dimensions and ↑ viability of infarcted tissue; No serious adverse events, including restenosis
Hill et al [64]	CCS ≥3, Severe reversible ischemia (Dobutamine MRI), LVEF 52±2,6%	G-CSF (16 pts) vs 15 healthy subjects	NA	NA	2 pts with NSTEMI and MI/death. No benefit in cardiac function
Jorgensen et al [65]	Onset of G-CSF treatment 30±12 hours after primary PCI	G-CSF treated (n=20) vs controls (n=21)	NA	NA	No difference between the 2 groups in in-stent neo-intimal hyperplasia or restenosis (IVUS) at 5 months invasive follow-up
Boyle et al	Severe reversible ischemia (SPECT) with CCS ≥3; LVEF 50± 4,7%	G-CSF (5 pts) vs 5 controls	Peripheral collected CD 34+ cells	ic	Angina ↓, collateral flow grade ↑ (angiography). No in-stent restenosis, but in pts with documented enhanced collaterals one with Acute Coronary Syndrome and one with Lentigo maligna of the scalp)
Huettmann et al [67]	Chronic severe HF, LVEF<30%	G-CSF treated DCM pts (n=7) vs G-CSF treated ICM pts (n=9) vs controls (ICM, n=8)	NA	NA	Improvement in NYHA class and 6MWT, echo unchanged; Occasional dyspnea, angina and 1 fatal Vfib (Treated ICM group)
Wang et al [68]	Severe reversible ischemia (SPECT) with CCS ≥3; LVEF 39±13%	G-CSF (n=13) vs controls (n=16)	NA	NA	Myocardial perfusion unchanged, LVEF (SPECT: no change, MRI: ↓) - Improvement in symptoms, NTG use. No adverse events
Kuethe et al [69]	Onset of G-CSF treatment PCI 7.6 ±5.2 hours after primary PCI	G-CSF treated (n=14) vs. controls (n=9)	NA	NA	No serious adverse events. ↑ regional wall motion, perfusion and LVEF (treatment group). 1/13 treated pts with in-stent restenosis (angiography)
Pompilio et al [70]	10 days -3 months after MI or large ischemic areas	4 pts (3 with MI treated with off-pump CABG, 1 with ischemia treated with transdiaphragmatic approach)	Peripheral collected CD133+ cells (mobilized with Lenogastrim)	Transepi-cardial	2 pts with follow-up (1 with perfusion, 1 with viability of the infarcted area); No serious adverse events

G-CSF, granulocyte colony-stimulating factor; PCI, percutaneous coronary intervention ; MNC, mononuclear cells; IRA, infarct-related artery; LVEF, left ventricular ejection fraction; CCS, Canadian Cardiovascular Society ; SPECT, single photon-emission CT; NTG, nitroglycerin; CABG; coronary artery bypass grafting; IVUS; intracoronary intravascular ultrasound; DCM, dilated cardiomyopathy; ICM, ischemic cardiomyopathy; NYHA, New York Heart Association; Vfib, Ventricular fibrillation; ic, intracoronary; LV, left ventricle; NSTEMI, Non-ST-elevation myocardial infarction

MOBILIZATION OF STEM/PROGENITOR CELLS

Based on the knowledge that bone marrow-derived progenitor cells can reach the target organ through peripheral blood[47,48] and that tissue repair is mediated by cytokines secreted from sites of ischemic injury[16,49-52], an effort was made to evaluate the enhancement of the natural repair mechanism by exogenous delivery of G-CSF (Granulocyte-colony-stimulating-factor) and/or other cytokines on cardiac repair.

Stem and progenitor cell mobilization with granulocyte colony-stimulating factor (G-CSF) and/or stem cell factor (SCF) and other cytokines has been shown to improve cardiac function after MI in the experimental setting, but results do not seem to be uniform in different species or even differing human populations. It also seems that the exact mechanism is still to be elucidated since early reports on differentiation of mobilized stem cells into cardiomyocytes and endothelial cells in the infarcted area are challenged by recent experimental work. These latter studies reported a direct antiapoptotic effect of G-CSF on cardiomyocytes and endothelial cells in infarcted hearts as well as an accelerated wound healing process in the necrotic tissue along with the myocardial regeneration effect[10,53-60].

For clinical purposes, G-CSF induced mobilization of stem cells offers a more practical and noninvasive approach but the increased rate of in-stent restenosis reported in the first clinical trial conducted (MAGIC trial) raised great scepticism on the safety of the method. However, the late FIRSTLINE-AMI and STEMMI trials reported a restenosis rate within the expected range. This was partly due to a different and more strict selection of patients in the latter studies regarding age, severity of coronary artery disease, chronicity of myocardial infarction and onset of subcutaneous G-CSF delivery after PCI of the IRA (Table 1)[61-70].

Our group has administered autologous selected CD 133+ stem/progenitor cells via the intracoronary route in 12 patients with an old, non-viable anterior wall MI with a LVEF lower than 40%[71,72]. These patients underwent echocardiographic and Tl-201 reinjection scintigraphic studies 4.4±2.0 months (mid-term) and 11.3±3.0 months (long-term) after BMSC delivery. We found a progressive and sustained benefit in myocardial perfusion as documented by an increase in perfusion ratio in the infarcted anterior wall and apex of the left ventricle. Perfusion ratio (calculated as the percentage of normalized radioactivity -counts/pixel- in the infarcted segment vs maximum Tl-201 uptake) increased during long-term follow-up by 22.6% ($p=0.005$) in the anterior wall and 34.7% in the apex ($p<0.001$). We also found a progressive and gradual effect on LV remodeling as manifested by a decrease in end-diastolic and end-systolic volumes of the left ventricle. Tissue Doppler imaging performed at 6 months after bone marrow transplantation revealed that local deformation increased significantly as indicated by the improvement of ejection time and maximum strain as well as peak systolic strain rate (Table 2).

SUMMARY AND CONCLUSION

Transplantation of stem cells for myocardial dysfunction after myocardial infarction is being intensively investigated for experimental as well as for clinical purposes. Contrary to the dogma that the heart is a terminally differentiated organ that cannot replace its own cell damage, there is now proof that the circulating blood provides the injured tissue with adult stem and progenitor cells, which have the potential to differentiate into multiple cell lineages and ultimately improve cardiac function. Many questions regarding the exact mode of action remain unanswered. The BOOST trial was the first completed, randomized study that showed safety, feasibility and efficacy of the method. The latest study published by Janssens and colleagues was the first double-blind, placebo-controlled study and failed to reveal an increase in global LV ejection fraction reported in the former study. Thus, it is imperative to conduct more double-blind randomized studies in order to evaluate this novel method and its exact role in the treatment of ischemic heart failure, but safety should always a primary concern.

TABLE 2. Baseline and Follow-up Echocardiographic Data

	Rest EDV (ml)	**Rest ESV** (ml)	**Rest LVEF** (%)	**Eet**	**Emx**	**PSySR**
Baseline	191.5±53.6	139.7±37.0	25.5±6.7	-7.55±3	-10.2±3.4	-0.7±0.23
Mid-term f/up	182.6±52.1	130.7±37.4	28.0±7.7	-	-	-
Long-term f/up	171.7±47.6	121.3±35.2	29.2±7.3	-11±5	-13.7±5.6	-1.1±0.24
p value	0.006	0.002	0.02	0.046	0.029	0.001

EDV, End-diastolic volume; ESV, End-sysolic volume; LVEF, Left ventricular ejection fraction; Eet= ejection time strain; Emx= maximum systolic strain; PsySR= peak systolic strain rate

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