PRIMEIRO USO DE CELULAS TRONCO PELA VIA RETROGRADA FOI REALIZADA NA ARGENTINA PELO PROF ROBERTO VIÑA NO MAIOR CENTRO DE PESQUISA COM CELULAS TRONCO DA AMERICA LATINA

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RESEARCH Open Access

Safety and feasibility of percutaneous retrograde coronary sinus delivery of autologous bone marrow mononuclear cell transplantation in patients with chronic refractory angina

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Abstract

Background: Chronic refractory angina is a challenging clinical problem with limited treatment options. The results of early cardiovascular stem cell trials using ABMMC have been promising but have utilized intracoronary or intramyocardial delivery. The goal of the study was to evaluate the safety and early efficacy of autologous bone marrow derived mononuclear cells (ABMMC) delivered via percutaneous retrograde coronary sinus perfusion (PRCSP) to treat chronic refractory angina (CRA).

Methods: From May 2005 to October 2006, 14 patients, age 68 +/- 20 years old, with CRA and ischemic stress-induced myocardial segments assessed by SPECT received a median $8.19*10^8 \pm 4.3*10^8$ mononuclear and $1.65*10^7 \pm 1.42*10^7$ $CD34^+$ cells by PRCSP..

Results: ABMMC delivery was successful in all patients with no arrhythmias, elevated cardiac enzymes or complications related to the delivery. All but one patient improved by at least one Canadian Cardiovascular Society class at 2 year follow-up compared to baseline ($p < 0.001$). The median baseline area of ischemic myocardium by SPECT of 38.2% was reduced to 26.5% at one year and 23.5% at two years ($p = 0.001$). The median rest left ventricular ejection fraction by SPECT at baseline was 31.2% and improved to 35.5% at 2 year follow up ($p = 0.019$).

Conclusions: PRCSP should be considered as an alternative method of delivery for cell therapy with the ability to safely deliver large number of cells regardless of coronary anatomy, valvular disease or myocardial dysfunction. The clinical improvement in angina, myocardial perfusion and function in this phase 1 study is encouraging and needs to be confirmed in randomized placebo controlled trials.

Background

An increasing number of patients with coronary artery disease remain symptomatic with disabling angina despite the optimal use of antianginal medications and percutaneous or surgical revascularization [1,2]. Therapeutic angiogenesis is an experimental strategy utilizing angiogenic proteins, gene therapy or stem cells for inducing neovascularization of chronically ischemic myocardium [3,4]. Currently, the majority of clinical studies investigating autologous bone marrow mononuclear cells (ABMMC) transplantation as a treatment for ischemic myocardium have been performed in patients with acute myocardial infarction using intracoronary delivery [5-11]. In contrast, in patients with refractory ischemia only a few trials have been published and all have used intramyocardial delivery [12-16]. Percutaneous retrograde coronary sinus perfusion (PRCSP) is a well-established technique for delivery of cardioplegia solution in cardiovascular surgery and for protection against myocardial ischemia in patients undergoing high risk percutaneous coronary intervention (PCI) [17-19]. Delivery by PRCSP has been shown to be a reasonable alternative to intracoronary and intramyocardial delivery in preclinical

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therapeutic angiogenesis models using both angiogenic proteins, gene therapy and stem cell therapy [20]. The technique has the potential advantages of safety delivering a larger number of cells with more homogenious delivery across the myocardium to patients with refractory angina despite the presence of severe underlying coronary artery disease, valvular disease or previous myocardial infarction which may complicate delivery by either intracoronary or intramyocardial approach. The aim of the present study was to evaluate the safety of ABMMC delivered into the ischemic myocardium via PRCSP in patients with chronic refractory angina (CRA).

Methods

Patients

Patients with Canadian Cardiovascular Society (CCS) class III-IV angina despite maximal medical or surgical therapy who were ineligible for further percutaneous or surgical revascularization (based on coronary anatomy) and who had evidence for reversible ischemia on an exercise single photon emission computed tomography (SPECT) were enrolled. A committee comprising two cardiovascular surgeons and two interventional cardiologists determined the ineligibility for percutaneous or surgical revascularization. Patients with acute myocardial infarction, percutaneous or surgical revascularization within six months of enrollment in the study, a history of malignant disease, severe renal dysfunction, or unexplained hematology or biochemical abnormalities were excluded. The local ethics committee at Clinica Maison de Sante and Centro Cardiovascular San Nicolá s approved the protocol and all patients gave informed consent. The review and analysis of the data was also approved by the IRB at the University of Utah.

Study protocol

The baseline screening assessment of patients included clinical evaluation, electrocardiogram (ECG), laboratory evaluation (complete blood count, blood chemistry, erythrocyte sedimentation rate, creatine kinase, and troponin T serum levels). Patients kept a record of daily angina frequency for three weeks, and the severity of angina was graded according to the CCS class at baseline, 3, 12, and 24 months. Within two weeks prior to cell therapy, exercise capacity was evaluated using bicycle ergometry in conjunction with SPECT imaging to assess myocardial ischemia and left ventricular (LV) function.

Periprocedural evaluation

Patients had complete blood count, creatine kinase levels and ECG performed immediately prior to and immediate after the procedure. Complete blood count, creatine kinase, and troponin T levels were also assessed eight hours post procedure with an ECG prior to discharge. All patients were monitored in the cardiac intensive care unit for 12 hours after the procedure and were discharged the following day. A transthoracic echocardiogram was performed prior to discharge to detect the presence of pericardial effusion. The clinical follow-up evaluations including the laboratory tests described above were performed 3, 12 and 24 months after the injection procedure. At 12 and 24 months of follow-up exercise bicycle testing, and gated SPECT were performed.

Assessment of exercise capacity

All patients performed a symptom-limited bicycle exercise test with a 20-W starting load and 10-W increments per minute at baseline and at 12 and 24 months after the injection procedure. Antianginal medication was continued. The test points were angina pectoris, physical exhaustion, dyspnea, significant decrease in blood pressure (10 mmHg), or achievement of maximal age-related heart rate. A 12-lead ECG was recorded before, during, and after the test. The total exercise duration, maximal workload achieved in percentages (expected for age, gender, height, and weigh) at baseline was compared to 12 and 24 months after ABMMC delivery.

Single-photon emission computed tomography

For the SPECT examination, a one-day rest-stress protocol was used. On the same day, images at rest were obtained 60 minutes after the $^{99\,m}$Technetium Sestamibi injection using a double-head SPECT camera as previously described. (17,18) Using the bicycle exercise test, $^{99\,m}$Technetium Sestamibi was injected intravenously at peak exercise (85% of the estimated heart rate) which was continued for 2 minutes after tracer injection and images were obtained 30 minutes after the stress test. Reconstruction yielded long-and short -axis projections perpendicular to the heart axis. The short-axis slices were displayed in polar map format.

Bone marrow aspiration and isolation of mononuclear cells

A volume of 300 ml of bone marrow was harvested from the iliac crest under local anesthesia and placed in heparinized Hanks' balanced salt solution. ABMMC were isolated by Hess 6% density gradient centrifugation with a final suspension volume of 50 ml and a mean cell concentration of $8.19*10^8 \pm 4.3*10^8$ mononuclear cells and $1.65*10^7 \pm 1.42*10^7$ CD34+ cells.

Delivery Procedure

The femoral vein was cannulated with a 7 French sheath, a 6 French catheter was placed in the coronary sinus and a 0.035 mm hydrophilic guide wire was placed in the interventricular or lateral vein followed by placement of a peripheral balloon into the mid portion of the coronary sinus to allow nonselective delivery of cells. (Cook Medical, Indiana, USA). The balloon was inflated at very low pressure (1 to 2 atm) for 10 minutes producing stagnation of the flow as previously described with infusion of the ABMMC.(18,19) 50 mls of ABMMC were injected manually through the balloon at a rate of 10 mls per minute. The average total procedure time for cell delivery was 30 minutes.(Figure 1)

Statistical analysis

Data are reported as median ± SDs. Quantitative data were compared using a paired, 2 tailed Student's test. Categorical data were compared using the Wilconxon signed rank test, a $p < 0.05$ was considered significant.

Results

The baseline clinical characteristics of the 14 enrolled RA patients are listed in Table 1. All patients had three vessel disease and were deemed ineligible for further revascularization by the screening committee of physicians. The patient's antianginal regimen was not changed during the 24 month follow-up.

Safety assessment

There was no evidence of inflammation or myocardial injury based on periprocedural laboratory evaluations (maximum erythrocyte sedimentation rate 20 ± 12 mm, maximum creatine kinase 137 ± 68 U/L, and maximum troponin T 0.010 ± 0.019 ng/mL) Ventricular arrhythmias were not observed during cell delivery or hospitalization and no patient had post procedural pericardial effusion by two-dimensional echocardiography before discharge. One patient died four months following the procedure due to liver cirrhosis, decompensated heart failure and pulmonary hypertension.

Clinical outcomes

The frequency of angina episodes per day decreased from 2.9 ± 3.9 at baseline to 1.0 ± 1.4 at 3 months ($p < 0.001$), 0.7 ± 1.2 at 12 months ($p < 0.001$) and 0.6 ± 1.2 at 24 months ($p < 0.001$). Angina improved in 13/14 patients and the mean Canadian Cardiovascular Society class improved from 3.2 ± 0.6 baseline to 1.9 ± 0.7 at 1 year ($p < 0.001$) and 1.8 ± 0.4 at 2 years ($p < 0.001$). The individual changes for anginal episodes per day for the 14 patients are shown in Figure 2.

Exercise capacity

At baseline, 14 patients had exercise duration time of 3.1 ± 1.4 minutes. This improved to 6.8 ± 1.0 and 7.4 ± 1.7 minutes at 12 and 24 months of follow-up respectively, ($p < 0.001$). The individual changes for exercise time are shown in Figure 3.

Myocardial perfusion by SPECT

The median percent of ischemic myocardium by SPECT was 38.2% at baseline and was reduced to 26.5% and 23.5% ($p = 0.001$) at 12 and 24 month follow-up (Table 2 and Figure 4). The number of segments with stress-inducible ischemia per patient improved 11.7% and 14.7% at 12 and 24 months of follow up. Examples of SPECT

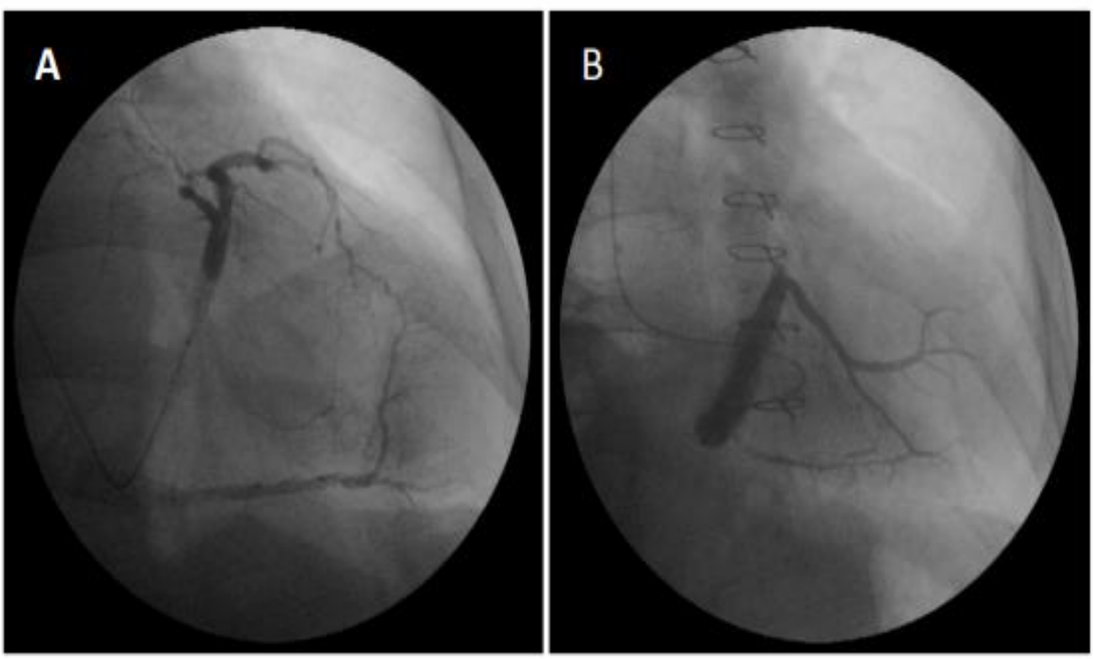

Figure 1 A: Unselected transplantation of BMC into the coronary veins, B: Selected transplantation of BMC into the lateral veins.

Table 1 Baseline Clinical Demographics

Demographic	ABMMC* (N = 14)
Median age	68
Female/Male ratio	1/6
Systemic Hypertension - N (%)	14 (100%)
Hyperlipidemia - N (%)	12 (85.7%)
Diabetes Mellitus - N (%)	4 (28.6%)
Previous myocardial infarction - N (%)	14 (100%)
Previous percutaneous coronary intervention - N (%)	4 (28.6%)
Previous coronary artery bypass surgery - N (%)	9 (64.3%)

*ABMMC = autologous bone marrow mononuclear cells

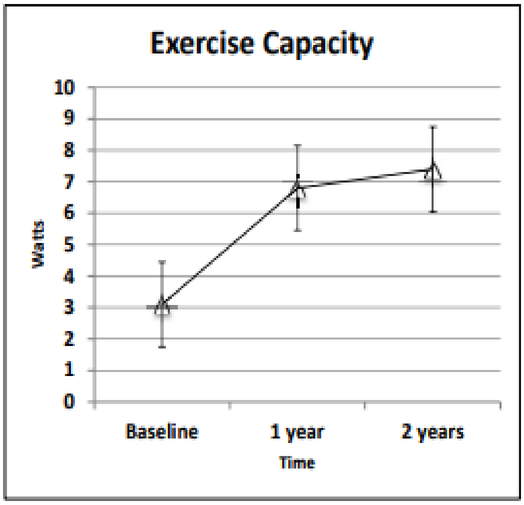

Figure 3 Exercise Capacity.

imaging of myocardial perfusion before and after ABMMC is shown for 2 patients in Figure 4A and 4B with improvements in both rest and stress perfusion.

LV Function assessed by SPECT

Median rest LV ejection fraction by SPECT at baseline was 31.2% and was improved to 35.4% and 35.5% at 12 and 24 month follow-up respectively ($p < 0.019$). (Table 2, Figure 5)

Discussion

This study demonstrates that ABMMC delivered by PRCSP in patients with CRA and stress-inducible ischemia was safe, may reduce anginal symptoms and improve exercise capacity. Stem cell transplantation by PRCSP is an alternative route of delivery to the myocardium for patients with coronary artery occlusion at their initial portions, or for patients with severe multivessel disease or occluded venous and arterial grafts. In this study we have demonstrated that, in patients with chronic ischemia with or without severely impaired LV function, that ABMMC injection enhances myocardial perfusion. In addition we observed that improved systolic LV function occurred only in patients with low ejection at baseline. Improvement and maintenance of the LV ejection fraction at 24 months follow-up was most likely a result of increased myocardial perfusion and global regional wall motion. This resulted in a reduced LV end-systolic volume. Consequently, the therapeutic effect appears to be more related to enhanced myocardial contractility rather than induction of LV reverse remodeling. The findings of the present study are consistent with the hypothesis that ABMMC promote angiogenesis, resulting in increased myocardial perfusion [18]. The present study was not designed to assess the underlying cellular mechanism of bone marrow cell injection improving myocardial perfusion and LV function. Therefore, secretion of pro-angiogenic factors by the bone marrow cells and differentiation of bone marrow cells in endothelium cells, smooth muscle cells, or cardiomyocytes could have contributed to the described effect. The mechanism of angiogenesis could be caused by differentiation of bone marrow cells in endothelial and/or vascular smooth muscle cells or by the production of angiogenic cytokines, as previously proposed but has not been fully validated [19]. ABMMC by the PRCSP has been proposed as a novel therapeutic option for patients with coronary artery disease. Until now most clinical studies investigating ABMMC were performed in patients with acute myocardial infarction. Data from patients with chronic ischemia are scarce. At present, only few studies in patients with chronic coronary artery disease have been published [9-13]. The current results are in line with studies on the safety and feasibility of intramyocardial delivery of ABMMC in patients with

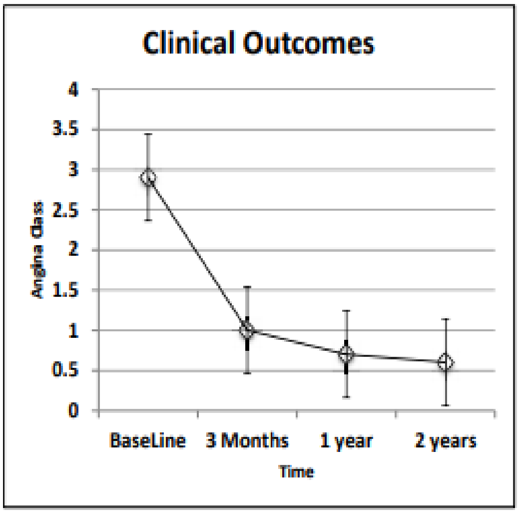

Figure 2 Clinical outcomes of angina class.

Table 2 Analysis pre and post ABMMC transplantation: Canadian Cardiovascular Society class, rest left ventricular ejection faction and ischemic myocardium percent

	BASELINE (N = 14)	1 YEAR (N = 13)	2 YEARS (N = 13)	P value
REST Global LVEF* (%) Median	31.2	35.4	35.5	0.019
CCS† Median	3	2	2	<0.001
Ischemic Myocardium (%) Median	38.2	26.5	23.5	0.001

*LVEF = left ventricular ejection fraction; †CCS = Canadian Cardiovascular Society

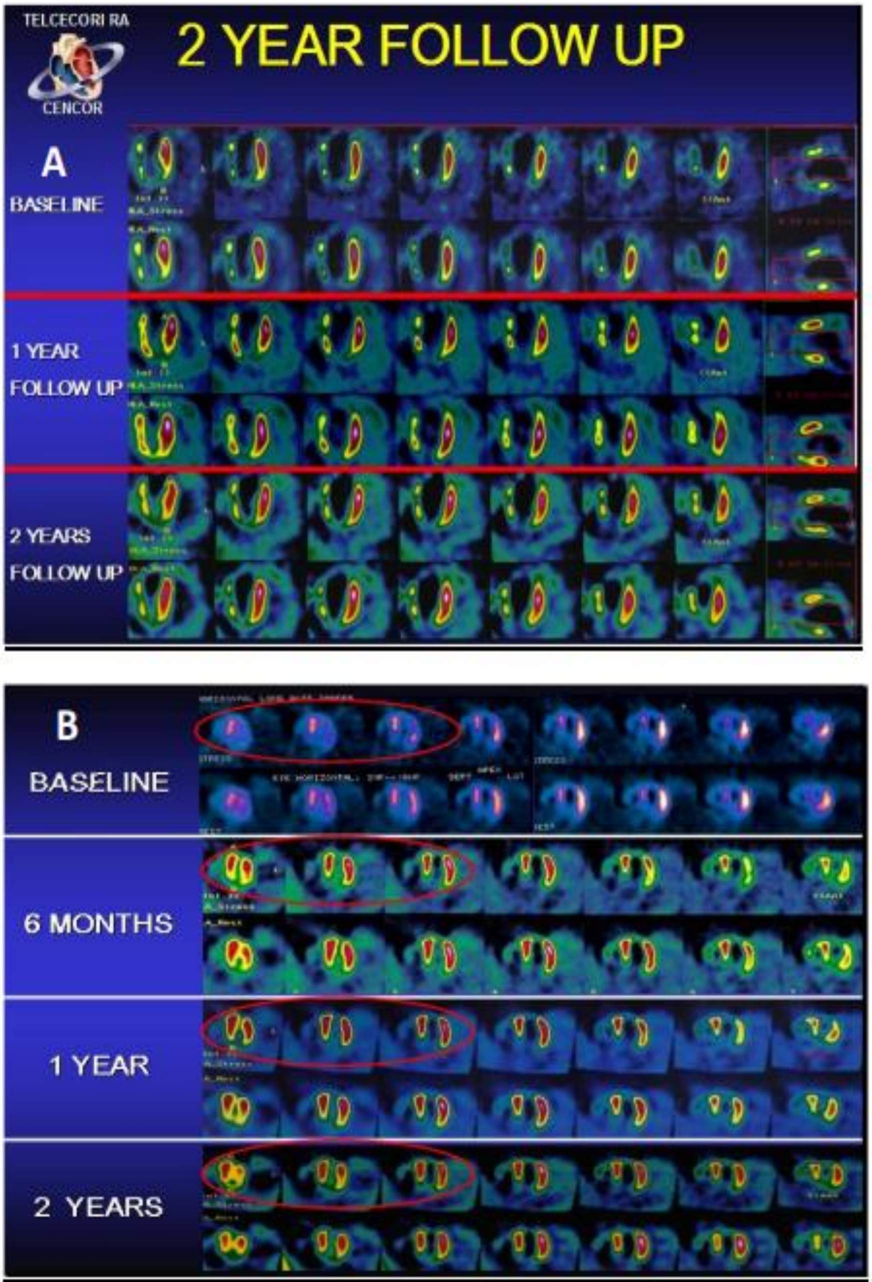

Figure 4 A: Perfusion imaging in a patient at 2 year follow up, B: Perfusion imaging in another patient at 2 year follow up.

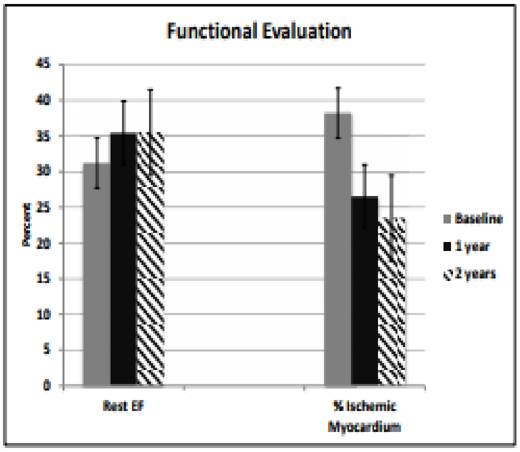

Figure 5 Functional evaluation of myocardium.

chronic ischemia and angina pectoris. Tse et al [9] described a reduction in anginal symptoms, improved wall motion and improved wall thickening at 3 'months follow-up in 8 patients. In addition, the area of hypoperfused myocardium was reduced on SPECT. Fuchs et al reported a reduction in anginal symptoms and improved myocardial perfusion in 27 patients with a trend toward improved LV ejection fraction [11]. Perin et al reported the same results in 21 patients [12]. In a randomized controlled study Losordo et al demonstrated data that CD34 + after GCSF can be safely transplanted via intramyocardial injection and may improve perfusion and reduce symptoms in patients with advanced coronary disease [14]. In this pilot trial ABMMC were successfully delivered in each patient without any major periprocedural events (i.e., death, myocardial infarction, ventricular arrhythmias, cardiac perforation, pericardial effusion or significant enzyme release) using PRCSP. The present study supports previously suggested beneficial effects in preclinical models of retrograde stem cells delivery for cardiac disease,[21,22] and the first to demonstrate improvement on symptoms, perfusion defects and LV function by PRVST in patients with preserved or decreased LV ejection fraction. However, we found similar improvements in patients with chronic ischemia with or without severely impaired LV function. This study suggests improvement and potential outcome durability of angina symptoms relief and better angina class, myocardial perfusion and contractility with this therapeutic approach in chronic refractory angina patients. We believe there may be clinical potential for this relatively novel method of cell delivery for patients suffering from refractory angina. We currently have a number of clinical trials using retrograde delivery of cells for both chronic refractory angina and heart failure based on this trial. Larger randomized trials will be needed to determine optimal cell numbers and to further understand the clinical outcomes.

Abbreviation List

ABMMC: autologous bone marrow mononuclear cells; CCS: Canadian Cardiovascular Society; CRA: refractory angina; ECG: electrocardiogram; LV: left ventricular; PRCSP: percutaneous retrograde coronary sinus perfusion; SPECT: single photon emission computed tomography.

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Authors' contributions

JT, RFV, AC, JC, CC, AC, JE, CY, JC enrolled and treated the patients. JT, RFV, AP conceived the study. JT, TH, AP all help to draft the manuscript and were involved in the data analysis. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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New Stem Cell Tactic Shows Promise for Heart Failure

Approach is 'simpler and probably safer' than earlier attempts, expert says

By Amy Norton
HealthDay Reporter
THURSDAY, July 30, 2015 (HealthDay News) -- A new method for delivering stem cells to damaged heart muscle has shown early promise in treating severe heart failure, researchers report.

In a preliminary study, they found the tactic was safe and feasible for the 48 heart failure patients they treated. And after a year, the patients showed a modest improvement in the heart's pumping ability, on average.

It's not clear yet whether those improvements could be meaningful, said lead researcher Dr. Amit Patel, director of cardiovascular regenerative medicine at the University of Utah.

He said larger clinical trials are underway to see whether the approach could be an option for advanced heart failure.

Other experts stressed the bigger picture: Researchers have long studied stem cells as a potential therapy for heart failure -- with limited success so far.

"There's been a lot of promise, but not much of a clinical benefit yet," said Dr. Lee Goldberg, who specializes in treating heart failure at the University of Pennsylvania.

Researchers are still sorting through complicated questions, including how to best get stem cells to damaged heart muscle, said Goldberg, who was not involved in the new study.

What's "novel" in this research, he said, is the technique Patel's team used to deliver stem cells to the heart. They took stem cells from patients' bone marrow and infused them into the heart through a large vein called the coronary sinus.

Patel agreed that the technique is the advance.

"Most other techniques have infused stem cells through the arteries," Patel explained. One obstacle, he said, is that people with heart failure generally have hardened, narrowed coronary arteries, and the infused stem cells "don't always go to where they should."

Researchers have also tried to directly inject stem cells into the heart during surgery or through a catheter. But only a limited number of cells can be safely injected that way, Patel said.

The new approach, he said, allows a bigger stem cell "dose."

According to the U.S. Centers for Disease Control and Prevention, more than 5 million Americans have heart failure -- a chronic, progressive condition where the heart cannot pump blood efficiently enough to meet the body's needs. Often, it arises after a heart attack damages the heart muscle.

Stem cells are primitive cells that can develop into different types of body tissue. Adults have stem cells in their bone marrow that give rise to blood cells. They also have small pools of stem cells within organs, including the heart.

Many researchers believe that if they can harness stem cells' natural capacity for repair, it could open a new option for advanced heart failure.

The current study, published July 27 in the journal *Stem Cells Translational Medicine*, is one of the latest efforts.
Patel's team recruited 60 patients with severe heart failure. They randomly assigned 48 to receive stem cell therapy along with their usual care, and 12 to stay with standard care only -- which typically involves medications that lower blood pressure, reduce strain on the heart and rid the body of excess fluid.

The stem cell therapy appeared safe, with no adverse effects linked to the treatment itself, the researchers say. And after one year, the patients showed a modest improvement in the heart's "ejection fraction" -- the amount of blood pumped out to the body with each contraction.

Goldberg said there are "theoretical advantages" to infusing stem cells via the coronary sinus. "It's simpler and probably safer," he said, than injections or infusions through heart arteries.

The big question, though, is does it work?

Scientists aren't even sure what stem cells do at the site of heart damage. Patel said it's unlikely that stem cells from the bone marrow magically become heart-muscle cells. Instead, they probably release hormones and other compounds that spur the growth of new blood vessels and help "remodel" the heart muscle.

Dr. Christopher O'Connor, editor-in-chief of the *Journal of the American College of Cardiology: Heart Failure*, agreed that the new technique might get more stem cells to the heart muscle.
The ultimate question, though, is whether any approach to stem cell therapy can change the course of heart failure, O'Connor said.

"We don't know if this will translate into clinically meaningful outcomes -- patients feeling better and living longer," O'Connor said. Still, he added, "the field is moving forward."

More information
California's Stem Cell Agency has a primer on stem cell basics.
SOURCES: Amit Patel, M.D., director, cardiovascular regenerative medicine, University of Utah, Salt Lake City; Lee Goldberg, M.D., associate professor, medicine, University of Pennsylvania Perelman School of Medicine, Philadelphia; Christopher O'Connor, M.D., director, Heart Center/Cardiovascular Clinical Services Unit, Duke University School of Medicine, Durham, N.C.; July 27, 2015 *Stem Cells Translational Medicine* online

Last Updated: Jul 30, 2015

New Method For Delivering Stem Cells To Damaged Heart Muscle Shows Promise In Treating Severe Heart Failure.

HealthDay (7/31, Norton, 5K) reports that "a new method for delivering stem cells to damaged heart muscle has shown early promise in treating severe heart failure...report" investigators. Researchers "took stem cells from" heart failure "patients' bone marrow and infused them into the heart through a large vein called the coronary sinus." The investigators found that "after a year, the patients showed a modest improvement in the heart's pumping ability, on average." Dr. Christopher O'Connor, editor-in-chief of the Journal of the American College of Cardiology: Heart Failure, is quoted as saying, "We don't know if this will translate into clinically meaningful outcomes – patients feeling better and living longer," but nonetheless, he added, "the field is moving forward."

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REVIVE Trial: Retrograde Delivery of Autologous Bone Marrow in Patients With Heart Failure

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Key Words. Heart failure • Cell transplantation • Coronary sinus • Retrograde approach

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ABSTRACT

Cell therapy is an evolving option for patients with end-stage heart failure and ongoing symptoms, despite optimal medical therapy. Our goal was to evaluate retrograde bone marrow cell delivery in patients with either ischemic heart failure (IHF) or nonischemic heart failure (NIHF). This was a prospective randomized, multicenter, open-label study of the safety and feasibility of bone marrow aspirate concentrate (BMAC) infused retrograde into the coronary sinus. Sixty patients were stratified by IHF and NIHF and randomized to receive either BMAC infusion or control (standard heart failure care) in a 4:1 ratio. Accordingly, 24 subjects were randomized to the ischemic BMAC group and 6 to the ischemic control group. Similarly, 24 subjects were randomized to the nonischemic BMAC group and 6 to the nonischemic control group. All 60 patients were successfully enrolled in the study. The treatment groups received BMAC infusion without complications. The left ventricular ejection fraction in the patients receiving BMAC demonstrated significant improvement compared with baseline, from 25.1% at screening to 31.1% at 12 months ($p = .007$) in the NIHF group and from 26.3% to 31.1% in the IHF group ($p = .035$). The end-systolic diameter decreased significantly in the nonischemic BMAC group from 55.6 to 50.9 mm ($p = .020$). Retrograde BMAC delivery is safe. All patients receiving BMAC experienced improvements in left ventricular ejection fraction, but only those with NIHF showed improvements in left ventricular end-systolic diameter and B-type natriuretic peptide. These results provide the basis for a larger clinical trial in HF patients. STEM CELLS TRANSLATIONAL MEDICINE *2015;4:1–7*

SIGNIFICANCE

This work is the first prospective randomized clinical trial using high-dose cell therapy delivered via a retrograde coronary sinus infusion in patients with heart failure. This was a multinational, multicenter study, and it is novel, translatable, and scalable. On the basis of this trial and the safety of retrograde coronary sinus infusion, there are three other trials under way using this route of delivery.

INTRODUCTION

Cardiovascular disease is the leading cause of death in the world [1, 2]. The overall mortality from coronary artery disease (CAD) has declined because of several factors, including improvements in pharmacologic therapy and revascularization techniques; in particular, reperfusion therapy with primary percutaneous coronary intervention [3]. These life-saving advances in the management of acute and chronic CAD have been associated with an increased prevalence of patients with heart failure (HF), with an estimated 250,000–350,000 patients who might be candidates for more advanced therapies [4]. HF, like all cardiovascular diseases, is highly age-dependent, and the prevalence will potentially double by 2030 with the advancing age of the U.S. and world populations [5].

The therapeutic options for patients who develop advanced HF are limited to heart transplantation or the use of ventricular assist devices [6]. This results in a large HF patient population with progressive symptoms and limited treatment options [7]. Biologically based cell and gene therapies for advanced HF have shown promise [8–11]. The goal of biological therapy, which uses the body's native repair mechanisms, is to reverse or restore the function of organs, tissues, and blood vessels. As such, it is ideal for HF patients. Many sources of stem cells are available for biological therapies, including abdominal fat [12], genetically engineered fibroblasts [13, 14], resident cardiac stem cells [15], human umbilical cords [16], and human endometrium [17]. However, the most widely used biological therapy has been autologous bone marrow for the treatment of cardiovascular disease [8, 11].

The route of delivery is an important variable in biologically based therapy. Direct epicardial injection at open heart surgery [18] and intracoronary infusion for acute myocardial infarction (MI) were

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the first methods described. Intracoronary delivery depends on inflammatory signals to recruit the cells to areas of injury and might be suboptimal in patients with advanced CAD or previous coronary artery bypass grafting. Therefore, catheters were developed to deliver biologic agents into the myocardium percutaneously using electrical mapping, fluoroscopy, or echocardiography to obtain precise localizations [19, 20]. Percutaneous, subendocardial, intramyocardial delivery is effective but carries risk, including perforation. In addition, patients with aortic valve disease, a thin myocardium, or left ventricular (LV) thrombus are not candidates. Both intracoronary and intramyocardial delivery are also limited in the number of cells that can be safely delivered. This has made translation from preclinical models to human dosing more challenging.

Retrograde venous delivery via the coronary sinus has been shown to be successful for cardioplegic solution delivery in open heart surgical procedures [21]. Preclinical models have demonstrated delivery efficacy equal to intramyocardial delivery. Additionally, a large number of cells can be delivered with this technique, and few patient contraindications exist. Tuma et al. demonstrated the safety of retrograde delivery in a study in which a large number of bone marrow cells were delivered retrograde to patients with chronic myocardial ischemia [22]. Based on their study results, we conducted a clinical trial to evaluate the safety and efficacy using the retrograde delivery of bone marrow-derived stem cells in patients with advanced HF.

Materials and Methods

Study Design

An institutional review board-approved prospective, randomized, multicenter, open-label study (ClinicalTrials.gov identifier NCT01299324) was conducted to assess the safety and feasibility of retrograde infusion of a concentrate of nucleated bone marrow cells via the coronary sinus in patients with congestive HF. All patients were deemed to have received optimal medical treatment by a HF specialist. The primary endpoints of the study were the safety and feasibility of using bone marrow aspirate concentrate (BMAC) delivered retrograde into the coronary sinus of patients with heart failure. The secondary endpoints were the safety of retrograde delivery, quality of life (QOL) measured by the Minnesota Living with Heart Failure questionnaire (MLWHFQ), cardiovascular symptoms measured by the New York Heart Association (NYHA) HF classification and the Canadian Cardiovascular Society (CCS) angina classification, B-type natriuretic peptide (BNP) levels, LV ejection fraction (LVEF) by echocardiography, and ischemia and LVEF measured by single photon emission computed tomography (SPECT) in the ischemic group. Additionally, the efficacy of retrograde BMAC delivery was compared with optimized standard of medical care directed by a heart failure cardiologist. All clinical adverse events were evaluated by the adjudication committee at the time of the incident, and a determination was made of whether the event was related or unrelated to the experimental treatment. The enrolled patients were stratified to ischemic and nonischemic groups. Within these groups, the patients were randomized electronically at enrollment to receive either BMAC via retrograde delivery or the medical standard of care in a 4:1 ratio. Only the patients randomized to receive BMAC underwent bone marrow aspiration and infusion. The patients randomized to the control group continued to receive the standard of care medical therapy as prescribed by a heart failure specialist. All imaging and follow-up information was blinded for the reviewers (i.e., echocardiography).

Patient Selection

The eligible patients were aged 18 years or older with congestive HF, an LVEF <40% by contrast echocardiography, NYHA class III or IV, stable with standard medical therapy for at least 1 month before screening, and had a life expectancy of 6 months or longer. The exclusion criteria included previous radiation treatment, bone marrow disorders, cirrhosis, bleeding/clotting disorders, acute MI less than 7 days from the treatment date, uncontrolled diabetes (hemoglobin A1c >9%), known active malignancy or risk of active malignancy (defined as abnormal PAP, chest radiograph, or mammogram findings or positive fecal hemoccult result), antibiotics within 7 days, high-dose steroids within 1 month before the procedure, dialysis, serum creatinine >3.0 mg/dl, and pregnancy.

Procedure

The patients in the BMAC group were taken to the catheterization laboratory, where both the harvest of bone marrow and the infusion of cells occurred during the same visit. Patients taking warfarin were corrected to an international normalized ratio of <1.6 at the time of the procedure. The patient's heart rate, blood pressure, and electrocardiographic (ECG) tracing were monitored throughout the procedure. Using a standard technique, a 240-ml sample of bone marrow was obtained from the posterior iliac crest. The sample was then processed and concentrated to a volume of 60 ml over 15 minutes using the Harvest Bone Marrow Aspirate Concentrate System (Harvest Technologies, Terumo BCT, Plymouth, MA, http://www.harvesttech.com). The Harvest BMAC system concentrates the entire nucleated cell population from the marrow aspirate and the platelets while reducing the number of red blood cells in the final product. It is a point-of-care system and concentrates the bone marrow at the patient's bedside. This concentrate was then drawn up into a series of sterile syringes for infusion into the coronary sinus.

The coronary sinus was accessed via the right femoral vein using a 7F 8-mm × 40-mm balloon catheter (Cook Medical Inc., Lafayette, IN, http://www.cookmedical.com) under fluoroscopic guidance. The proper catheter position and balloon occlusion were confirmed with fluoroscopy. The prepared cell concentrate of 60 ml was infused continuously over a 5-minute period. The balloon remained inflated for 10 minutes after infusion to permit the migration of the cells into the cardiac tissue. After this dwell time, the balloon was deflated and the catheter removed. Local hemostasis was obtained, and the patient was transferred to a telemetry monitored unit for 24 hours of observation. The average time to perform the cell delivery from venous access to catheter removal was 29 ± 14 minutes.

Follow-Up

The patients returned for follow-up visits at 1, 3, 6, and 12 months. Cardiac biomarkers and chemistry and hematological laboratory tests were collected at their initial screening and subsequent 3-, 6-, and 12-month follow-up visits. For the BMAC patients, laboratory tests were obtained both before and after the procedure, including markers of cardiac injury, troponin I and creatine phosphokinase, and the complete blood count and renal function tests. Adverse events were recorded at each visit. SPECT scans were obtained at baseline and at 6 months in the ischemic group to assess LV ischemia and function. Echocardiography, ECG, and the MLWHFQ were performed at the 3-, 6-, and 12-month follow-up visits.

Statistical Analysis

Study data were collected using a dedicated electronic data capture system prospectively with real-time queries. Patient

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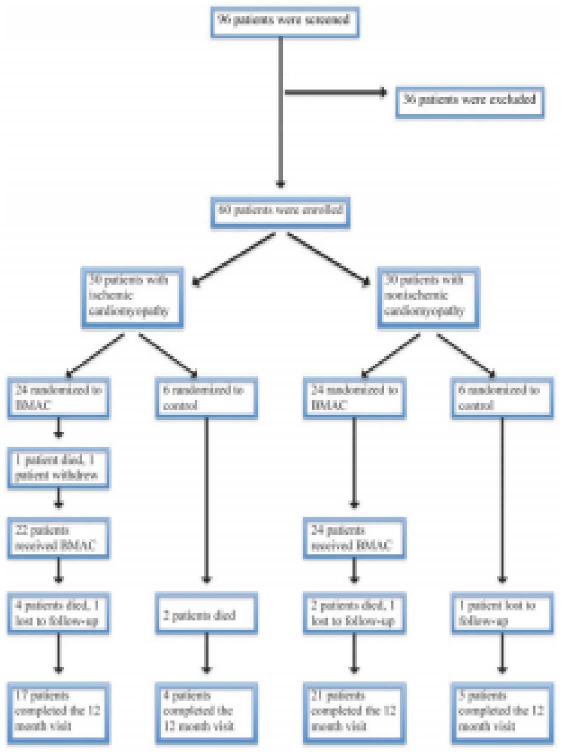

Figure 1. Patient disposition. Patients with heart failure were stratified by the presence of ischemic or nonischemic disease. Within each stratification, the patients were randomized to BMAC or control in a 4:1 ratio. The numbers of patients randomized, treated, and followed for 12 months are shown for each study arm. Abbreviation: BMAC, bone marrow aspirate concentrate.

Quality of Life

In general, the BMAC-treated patients did not progress to worsening NYHA classifications from baseline to the 12-month follow-up visit. All patients were in NYHA class 3 or 4 at the time of screening. At the 12-month follow-up visit, 8, 10, and 3 patients were in NYHA class 1, 2, and 3 in the nonischemic BMAC group and 3, 1, and 1 patient in the ischemic control group were in NYHA class 2, 3, and 4, respectively. In the ischemic BMAC group, 6, 9, 1, and 1 patient were in NYHA class 1, 2, 3, and 4 and 1, 1, and 2 patients in the ischemic control group were NYHA class 1, 2, and 3, respectively. The progression in health status as measured by the NYHA classification among the nonischemic BMAC patients was better than that in the nonischemic control group at all time points. A similar, although less pronounced, trend was seen in the ischemic stratification. The progression of NYHA status is shown in Figure 2A, 2B. The changes seen in NYHA class were not significant. The MLWHFQ scores improved from screening to 6 months among the BMAC and control patients in the ischemic and nonischemic stratifications both, with no significant different between groups. The CCS scores improved minimally in the nonischemic stratification, with 24 of 27 patients in class 1 at screening, and 27 of 27 in class 1 at 12 months. In the ischemic stratification, the CCS class worsened in the BMAC group, with an initial median CCS of 1 and a median of 2 at 12 months. In the ischemic control group, the distribution of CCS scores did not change.

Objective Measures

In the ischemic stratification, 22 of the 24 BMC group patients had an elevated BNP level at screening. In the control group, all 6 patients had elevated BNP. In the nonischemic stratification, 20 of 24 patients in the BMAC group had elevated BNP, and 5 control group patients had elevated BNP. BNP decreased in the nonischemic BMAC group. For this group, the average change in BNP from before infusion was −21.9, −26.2, −47.7, and −100.7 pg/ml at discharge and 3, 6, and 12 months respectively, compared with the nonischemic control group with BNP changes of +118.6, −162.6, and +24.0 pg/ml at 3, 6, and 12 months, respectively. In the ischemic stratification, the BNP changes in the BMAC group were −34.1, −12.11, +417.6, and +182.5 pg/ml at discharge and 3, 6, and 12 months compared with −355.7, −240.0, and −234.4 at 3, 6, and 12 months in the control group, respectively. The ischemic control group had very high BNP levels at baseline, nearly double that of the other groups. Changes in BNP between the BMAC and control groups did not reach statistical significance in either the ischemic or nonischemic stratification (Fig. 2C, 2D).

The mean ejection fraction in the BMAC groups in both the ischemic and the nonischemic stratifications demonstrated significant improvement compared with baseline screening by contrast echocardiography. The EF improved from 25.1% at screening to 31.1% at 12 months ($p = .007$) in the nonischemic group. The mean EF in the ischemic control group also demonstrated significant improvement compared with baseline, from 25.0% at screening to 33.1% at 12 months ($p = .006$; Fig. 2E, 2F). In both the ischemic and the nonischemic stratifications, no significant differences were seen between the mean EF in the BMAC and control groups at 12 months ($p = .814$ and $p = .954$, respectively).

A significant decrease was seen in the LV end-systolic diameter (LVESD) as measured by echocardiography in the BMAC group of the nonischemic stratification, which during the 12-month period changed from 55.6 mm to 50.9 mm ($p = .020$). A similar result was not seen in the ischemic BMAC group, with the LVESD changing from 51.7 mm to 51.6 mm at 12 months ($p = .0692$). No significant changes were seen in the end-diastolic diameter in either stratification. (Fig. 2G–2J).

SPECT was performed at the screening visit and at the 6-month follow-up visit only in the patients in the ischemic stratification. No significant change was seen in LVEF or perfusion in either the control or BMAC groups at the 6-month follow-up visit. The LVEF in the ischemic BMAC group changed from a mean of 27.09% ± 7.46% to 29.29% ± 6.74% at 6 months and 23.33% ± 10.39% to 31.25% ± 14.24% in the ischemic control group.

Table 2. Serious adverse events by stratification and treatment group

	Nonischemic stratification		Ischemic stratification	
Serious adverse events	BMAC (n = 24)	Control (n = 6)	BMAC (n = 22)	Control (n = 6)
Cardiac death	2 (8.3)	0	3 (13.6)	2 (33)
Noncardiac death	0	0	2 (9.1)	0
Ventricular arrhythmia	1 (4.2)	0	0	0
CHF exacerbation	0	1 (16.7)	2 (9.1)	0

Data are presented as n (%).
Abbreviations: BMAC, bone marrow aspirate concentrate; CHF, congestive heart failure.

Discussion

The present study is the first randomized trial using retrograde coronary sinus delivery of bone marrow mononuclear cells in HF patients. The trial has demonstrated both the safety and

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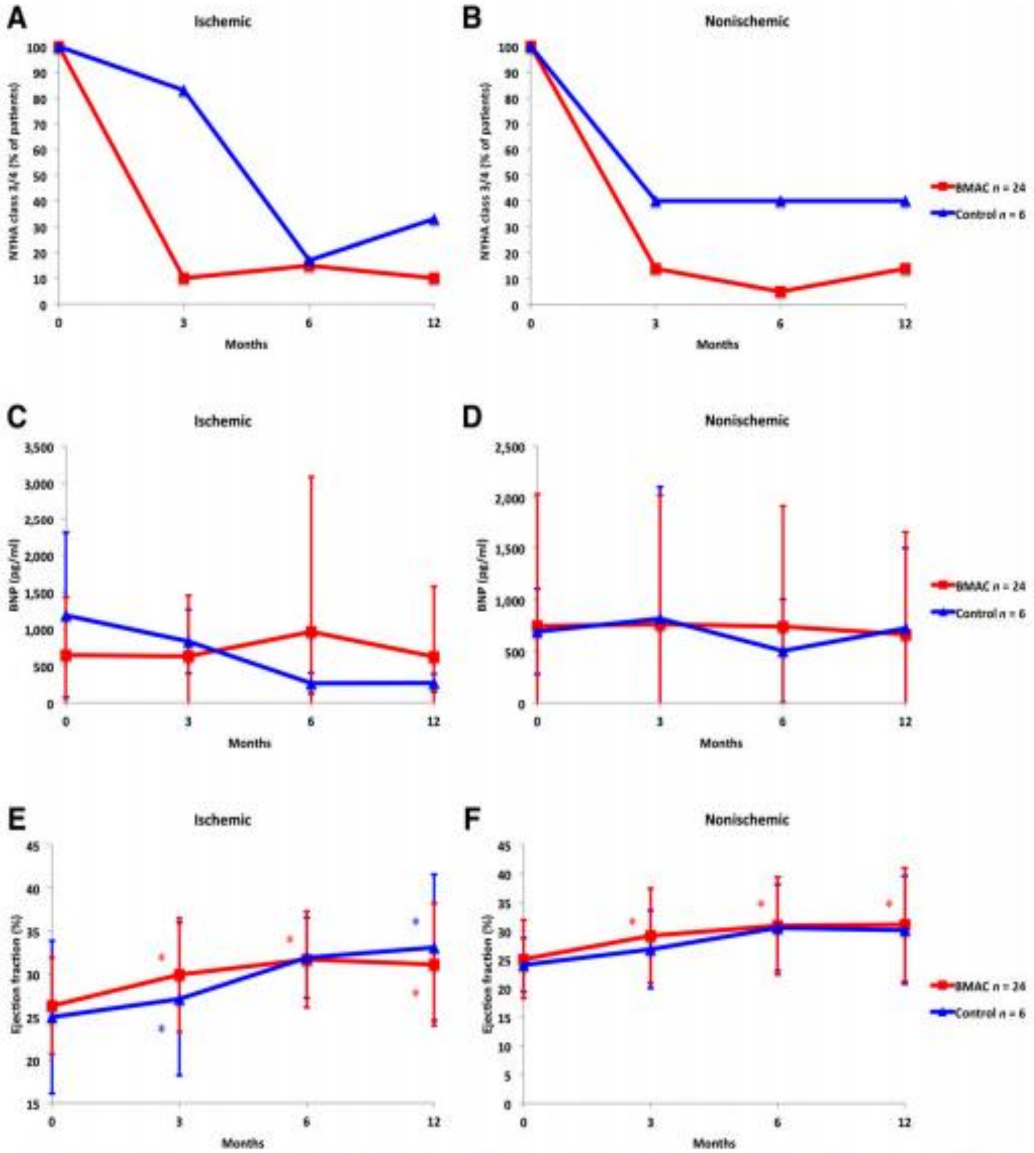

Figure 2. Cardiovascular parameters. The cardiovascular parameters for both ischemic and nonischemic cardiomyopathy patients in the BMAC and control groups. The percentage of BMAC (red squares) or control (blue triangles) patients with a NYHA class score of III or IV is shown for ischemic **(A)** and nonischemic **(B)** patients. **(C, D):** BNP in ischemic **(C)** and nonischemic **(D)** patients. **(E, F):** Ejection fraction in ischemic **(E)** and nonischemic **(F)** patients. **(G, H):** Left ventricular end-diastolic diameter in ischemic **(G)** and nonischemic **(H)** patients. **(I, J):** Left ventricular end-systolic diameter in ischemic **(I)** and nonischemic **(J)** patients. Results are presented as mean and standard deviation. *, $p < .05$, statistically significant compared with baseline. Abbreviations: BMAC, bone marrow aspirate concentrate; BNP, B-type natriuretic peptide; NYHA, New York Heart Association.

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the feasibility in patients with NYHA class 3 or 4 HF. Preclinical studies have demonstrated that total occlusion of the coronary sinus for up to 60 minutes is safe owing to the presence of the Thebesian veins, which also drain cardiac venous blood. This allows biologic agents to be infused retrograde under low pressure into the great and middle cardiac veins. Zakharova et al. also recently demonstrated the preclinical feasibility of retrograde cell delivery with distribution throughout the left ventricle [23]. Retrograde delivery of cells was developed to minimize the risks associated with cardiac cell delivery. Most endocardial, epicardial, and intracoronary delivery techniques have limitations such as myocardial wall thickness, invasiveness, or coronary blockage that would cause perforation or poor cell distribution. The number of cells delivered by these techniques is limited by microclumping, leading to infarcts. Thus far, cell doses of no greater than 300 million cells have been safely used owing to these issues. Retrograde delivery enables a larger cell dose, which is translatable based on most preclinical models [24]. The optimal retention of cells remains an ongoing investigation. Thus, retrograde coronary sinus delivery was chosen for the cell delivery route in the present trial.

Although adverse events occurred in all treatment arms, most were classified as unrelated to the procedure and should not raise safety concerns for this procedure. The rate of death in these patients should be considered a function of the poor health of this patient population that we aim to improve with this procedure, rather than a function of the procedure or cell infusion itself.

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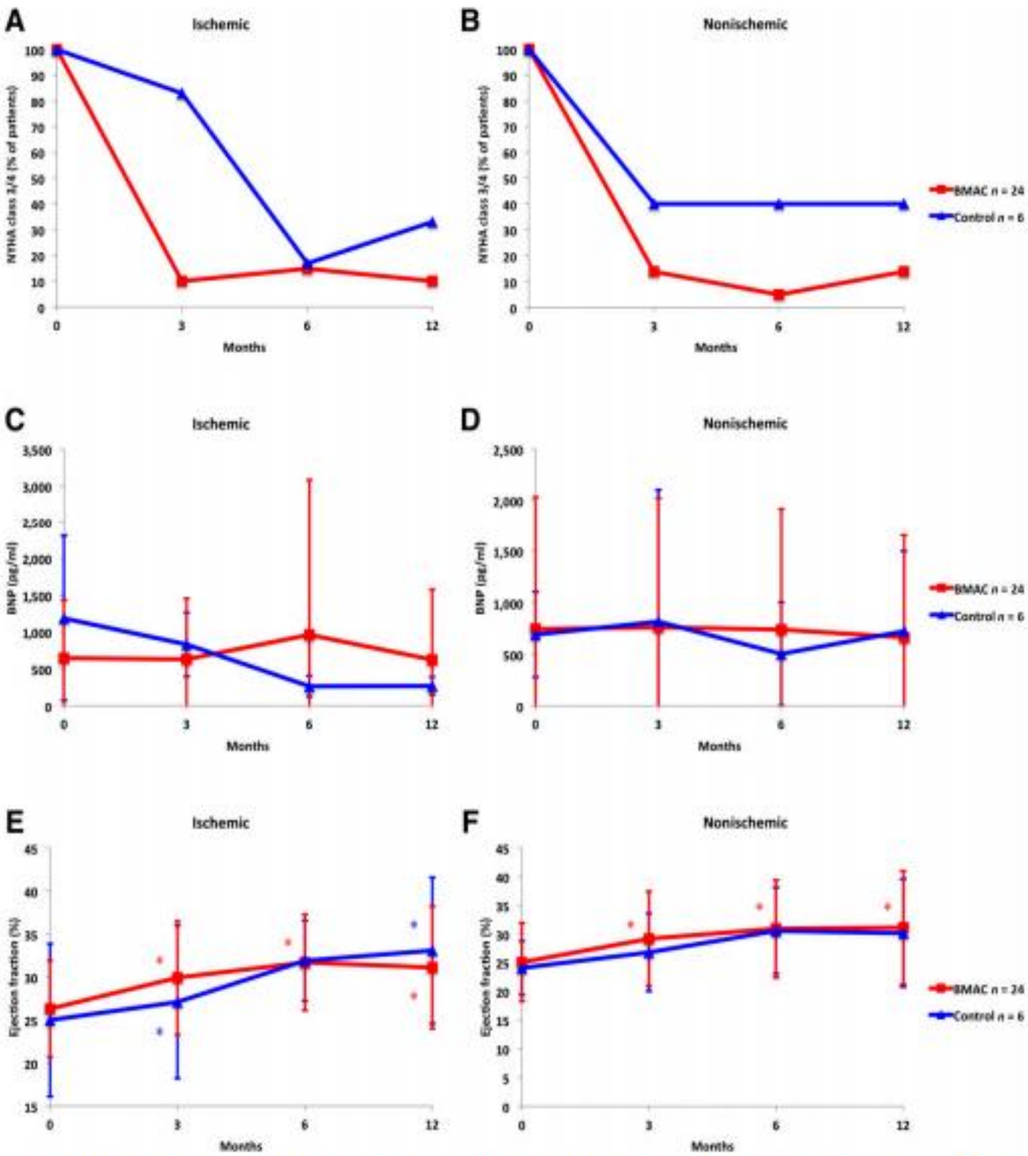

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labeled. However, the findings should lead to larger studies to further evaluate the potential benefits of BMAC in patients with ischemic and nonischemic cardiomyopathy.

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Author Contributions

A.N.P.: conception/design, provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript; S.M., G.T., H.I., and N.T.: provision of study material or patients, collection and/or assembly of data, data analysis and interpretation, final approval of manuscript; A.A.W. and T.D.H.: data analysis and interpretation, manuscript writing, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

A.N.P. has compensated honoraria from Cook Medical Inc. The other authors indicated no potential conflicts of interest.

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