

Cell-Based Therapy for Myocardial Dysfunction After Fontan Operation in Hypoplastic Left Heart Syndrome

Muhammad Y. Qureshi, MBBS; Allison K. Cabalka, MD; Shakila P. Khan, MD; Donald J. Hagler, MD; Dawit T. Haile, MD; Bryan C. Cannon, MD; Timothy M. Olson, MD; Susana Cantero-Peral, MD, PhD; Allan B. Dietz, PhD; Darcie J. Radel, MT; Nathan W. Taggart, MD; Angela M. Kelle, MD; Vilmarie Rodriguez, MD; Joseph A. Dearani, MD; and Patrick W. O'Leary, MD; from the Wanek Program Regenerative Medicine Pipeline

Abstract

Myocardial dysfunction after Fontan palliation for univentricular congenital heart disease is a challenging clinical problem. The medical treatment has a limited impact, with cardiac transplant being the ultimate management step. Cell-based therapies are evolving as a new treatment for heart failure. Phase 1 clinical trials using regenerative therapeutic strategies in congenital heart disease are ongoing. We report the first case of autologous bone marrow–derived mononuclear cell administration for ventricular dysfunction, 23 years after Fontan operation in a patient with hypoplastic left heart syndrome. The cells were delivered into the coronary circulation by cardiac catheterization. Ventricular size decreased and several parameters reflecting ventricular function improved, with maximum change noted 3 months after cell delivery. Such regenerative therapeutic options may help in delaying and preventing cardiac transplant.

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Staged surgical palliative procedures culminating in a Fontan circulation represent the standard of care for most patients with functionally single ventricles, such as those with hypoplastic left heart syndrome (HLHS). Long-term complications of the Fontan procedure include myocardial dysfunction secondary to multiple factors including prolonged increased workload, leading to heart failure requiring cardiac transplant in many cases.¹ With the growing population of patients with Fontan palliation and the relatively unchanged limited availability of organs for transplant, it is imperative that alternative treatment strategies be sought. Medical treatment of heart failure with afterload-reducing agents and other medications used in structurally normal myopathic hearts has not shown clear benefit in these

patients. Cell-based therapies, primarily using bone marrow–derived cells, have been tested in clinical trials for adults with ischemic heart disease²⁻²² and nonischemic dilated cardiomyopathy²³⁻²⁵ for many years. These studies are now being applied in children and adults with congenital heart disease. However, the utility, safety, and efficacy of cell-based therapies are not established in these patients. We have initiated a phase 1 clinical trial (NCT 02549625) for use of bone marrow–derived mononuclear cells (BM-MNCs) in Fontan procedure–treated patients with myocardial dysfunction. The mononuclear cell preparation is a heterogeneous mixture of cells containing CD34 progenitor stem cells thought to promote myocardial regeneration through paracrine mediators. We report the first case of intracoronary delivery of autologous BM-MNCs for single

From the Division of Pediatric Cardiology (M.Y.Q., A.K.C., D.J.H., B.C.C., T.M.O., N.W.T., A.M.K., P.W.O.), Division of Pediatric Hematology and Oncology (S.P.K., V.R.), Division of Pediatric Anesthesia (D.T.H.), Department of General Internal Medicine (S.C.-P.), Division of Transfusion Medicine (A.B.D., D.J.R.), and Division of Cardiovascular Surgery (J.A.D.), Mayo Clinic, Rochester, MN.

right ventricular dysfunction after Fontan palliation.

REPORT OF CASE

The patient presented for enrollment in our cell therapy clinical trial at age 25 years with clinical heart failure symptoms. He was born with HLHS (mitral and aortic valve stenosis, severely hypoplastic left ventricle, and ascending aorta). His surgical history consisted of Norwood operation at age 1 month followed by hemi-Fontan procedure at age 4 months, and finally creation of a nonfenestrated, lateral tunnel-type Fontan connection at an early age (18 months). At age 20 years, he began to experience dyspnea with activity that progressed over the next 2 years. One year after the onset of symptoms, obvious ascites developed. He did not have enteric protein loss or hypoalbuminemia but was found to have severe regurgitation of the neo-aortic valve and severe right (systemic) ventricular systolic dysfunction. He underwent a number of procedures and trials of medical therapy, including embolization of aortopulmonary collaterals, catheter dilation/stenting of the left lower pulmonary vein and left pulmonary artery, and a course of intravenous inotropic support, with no lasting change in symptoms or ascites. He was evaluated for cardiac transplant because he was not a candidate for surgical replacement of neo-aortic valve due to severe ventricular dysfunction with estimated ejection fraction (EF) of 15%. Evaluation revealed marked prior sensitization to human leukocyte antigens (85% unacceptable antigen profile), creating a prohibitive risk for cardiac transplant. Medical treatment was subsequently optimized with multiple drugs including enalapril, carvedilol, digoxin, and diuretics. The doses were escalated over several months to the maximum tolerated by the patient. He had resolution of most of his symptoms within a period of 4 to 6 months. He remained clinically stable over the next 2 years. However, his right ventricle (RV) remained severely dilated because of persistent neo-aortic regurgitation, and ventricular EF remained moderately decreased at 30% to 35%. Despite these improvements, the risk of aortic valve replacement was still felt to be prohibitive. Transcatheter neo-aortic valve replacement was not possible because of the massively dilated neo-aortic root, which was larger than any available device. Pacemaker therapy was

not attempted as an isolated approach because the risk of epicardial placement was felt to outweigh the benefit of restoring heart rate response in the setting of his dysfunction and previous cardiac operations.

At age 25 years, the patient was enrolled into the phase 1 clinical trial using BM-MNCs in patients with myocardial dysfunction after Fontan procedure (NCT 02549625). Preenrollment screening revealed an increased N-terminal pro-B-type natriuretic peptide (NT-proBNP) level of 503 pg/mL (normal, <51 pg/mL), with a normal creatine kinase-MB (CK-MB) level and undetectable cardiac troponin T. Baseline C-reactive protein was normal. Holter monitoring revealed sinus bradycardia (heart rate range, 32-69 beats/min) with multiple pauses, the longest being 3.11 seconds. There was a short 10-beat run of supraventricular tachycardia at a rate of 111 beats/min and occasional (<1%) ventricular and supraventricular ectopic beats. Transthoracic echocardiography revealed severe RV enlargement with estimated EF of 30% to 35%. Cardiac magnetic resonance (CMR) imaging identified an RV end-diastolic volume of 213 mL/m² and calculated RV EF of 34%. The origins and proximal courses of the coronary arteries were normal on CMR imaging.

Under deep conscious sedation and local anesthesia, 75 mL of bone marrow was aspirated from the iliac spine and collected in a heparinized collection bag. The bone marrow aspirate was transported to the Human Cell Therapy Laboratory at Mayo Clinic, where it was stored at refrigerated temperature (2°C-8°C) and processed to obtain the mononuclear cell fraction using a Ficoll density gradient closed system the following day. The final dose of 2×10^6 cells/kg (94% viability) was suspended in 12 mL of 2.5% human albumin in Plasma-Lyte A. The Gram stain was negative and cultures (aerobic and anaerobic) remained negative for 14 days.

Cardiac catheterization was performed after the cells were processed and passed quality control. Bivalirudin was used for anticoagulation during the procedure instead of heparin to avoid potential disruption of cell migration and engraftment.²⁶ Hemodynamic assessment revealed a mean pressure of 16 mm Hg in Fontan circuit and a transpulmonary gradient of 1 mm Hg, both of which were normal.

The RV end-diastolic pressure was moderately increased to 16 mm Hg with no significant gradient across the neo-aortic valve or the aortic arch. The calculated cardiac index by Fick's principle was reduced at 1.7 L/min per m² (normal, 2.5-4.0 L/min per m²). Angiography revealed no evidence of coronary artery disease. The cell product was administered within 3 hours of completion of the BM-MNCs preparation. Two million total nucleated cells per kilogram were dispensed in a 12-mL volume. Six milliliters of the cell product was infused in the right coronary artery over 10 seconds without interrupting blood flow. The catheter was maintained in position for an additional 20 seconds after the infusion was completed, administering a 1- to 2-mL flush of sterile saline to deliver the full dose of cells within the "dead space" of the catheter while avoiding washing them out of the coronary vasculature. The same process was repeated for the left coronary artery, in accordance with the study protocol. Electrocardiographic monitoring did not reveal any ST-segment or T-wave changes during the procedure. The patient tolerated the procedure well with no evidence of acute complications or adverse effects.

After cell infusion, the patient remained in a monitored unit for 24-hour telemetry. Troponin T was undetectable at 3, 6, and 24 hours after cell delivery. The CK-MB level at 24 hours remained normal. The NT-proBNP level was elevated to 969 pg/mL at 24 hours. Glucose levels were checked every 2 hours and remained normal for 24 hours. A fever to 39.8°C developed 18 hours after cell delivery, with elevated C-reactive protein to 70.8 mg/L (normal, <8 mg/L) but a normal leukocyte count. Empiric antibiotics were not started. The patient was discharged from the hospital and seen in the clinic the following day, with resolution of fever. Blood cultures remained negative after 14 days.

Scheduled follow-up visits occurred at 1, 3, and 6 months after cell delivery. At the first visit, the C-reactive protein and NT-proBNP levels had returned to baseline levels. The patient reported one episode of palpitations, which resolved spontaneously. Holter monitoring at that visit revealed 2 short runs of supraventricular tachycardia (maximum 4 beats). Ventricular and supraventricular ectopic beat

| Echocardiographic parameters | Baseline | 1 mo | 3 mo | 6 mo |
|---------------------------------|----------|------|------|-------|
| Estimated RV EF (%) | 30-35 | 35 | 45 | 35-40 |
| RV area analysis | | | | |
| Apical areas | | | | |
| Diastole (cm ²) | 51.5 | 52.8 | 52.7 | 52.4 |
| Systole (cm ²) | 32.8 | 32.6 | 33.0 | 33.4 |
| Apical FAC (%) | 36.3 | 38.2 | 37.4 | 36.0 |
| Short-axis areas | | | | |
| Diastole (cm ²) | 53.7 | 53.4 | 47.8 | 48.8 |
| Systole (cm ²) | 40.5 | 34.4 | 25.4 | 28.1 |
| Short-axis FAC (%) | 24.6 | 35.6 | 46.9 | 42.4 |
| Biplane FAC (%) | 30.5 | 36.9 | 42.2 | 39.3 |
| TAPSE (cm) | 10.7 | 14.0 | 20.0 | 16.0 |
| RV MPI | 0.59 | 0.75 | 0.61 | 0.63 |
| TVS' | 0.07 | 0.08 | 0.1 | 0.07 |
| Myocardial deformation analysis | | | | |
| Apical longitudinal strain | -17 | -16 | -19 | -16 |
| Circumferential strain | -7 | -17 | -16 | -19 |
| Radial strain | 25 | 36 | 18 | 28 |

EF = ejection fraction; FAC = fractional area change; MPI = myocardial performance index; RV = right ventricle; TAPSE = tricuspid valve annular plane systolic excursion; TVS = tricuspid valve annular systolic velocity.

frequency remained less than 1%. Subsequently, the patient reported no cardiac symptoms and no additional palpitations. All blood test levels remained at the baseline levels. Holter recording at the 3- and 6-month visits were unrevealing. By the final 6-month follow-up visit, the patient had started playing basketball and reported a greater activity level than before the cell infusion. The NT-proBNP level had increased to 1094 pg/mL, whereas the CK-MB level and the troponin T level remained normal. No medication changes were made during the study period.

Echocardiography was performed at each of the 3 follow-up visits, and detailed CMR imaging was obtained 6 months after cell infusion. Results from these scans are summarized in Tables 1 and 2. Both modalities revealed a favorable decrease in ventricular size. Visually estimated EF increased from 30% to 35% to 45% at 3 months but decreased to 35% to 40% at the 6-month visit. Biplane fractional area change improved from 31% at baseline to 39% at 6 months (normal, >35%). The maximal fractional area change value of 42% was observed 3 months after cell infusion. Improved myocardial function was also evident by measurement of tricuspid annular plane systolic excursion and circumferential

myocardial strain, which revealed maximal increase at the 3-month follow-up time point. Tricuspid annular plane systolic excursion declined modestly, but RV longitudinal strain had decreased to baseline at 6 months. Little change was detected in systolic annular tissue Doppler velocity, myocardial performance index, and radial strain.

The CMR scan at the 6-month visit revealed a 10% reduction in RV diastolic volume (Table 2) and an increase in calculated EF from 34% to 38%. Myocardial deformation analysis performed on the CMR images illustrated improvement in RV longitudinal, circumferential, and radial strain values from baseline.

Two months after the last study visit, the patient underwent neo-aortic valve replacement with an On-X 27/29 mechanical prosthesis, reduction neo-ascending aortoplasty, and placement of an epicardial pacing system for sinus bradycardia with frequent long pauses. Intraoperative transesophageal echocardiography after valve replacement revealed the estimated RV EF to be 25% to 30%. The patient tolerated the procedure very well and was discharged home on the eighth postoperative day. The discharge echocardiography illustrated improved RV systolic function relative to the immediate postoperative transesophageal echocardiography with an estimated RV EF of 40%.

DISCUSSION

This case describes the first patient with HLHS to receive intracoronary infusion of autologous BM-MNCs for RV dysfunction after Fontan palliation. The primary objective of the clinical

trial was safety and feasibility of intracoronary delivery of BM-MNCs. The case highlights these aspects, as well as the secondary objective of efficacy. The patient tolerated bone marrow harvest and the intracoronary delivery of BM-MNCs by cardiac catheterization without complications. The only observed adverse event was fever and elevated C-reactive protein, which resolved spontaneously and were attributable to an inflammatory response to the delivered cells. The RV size decreased and most measures of systolic function improved with maximal change observed at the 3-month follow-up time point. A slight decline in ventricular function was noted over the next 3 months. However, almost all parameters had improved at the end of the study compared with baseline.

The proposed mechanism of action of cell-based therapy is the paracrine effect of mononuclear cells on the surrounding myocardium. The infused cells adhere to and cross the coronary artery walls (margination) and migrate into the myocardial tissue. The BM-MNCs are expected to survive only a few weeks after infusion. However, there is an increasing body of evidence supporting the hypothesis that paracrine mechanisms mediated by a broad variety of cytokines, growth factors, and chemokines released by these cells play an essential role in creating the regenerative potential in the surrounding myocardium.²⁷⁻³⁰ These mediators favorably impact cardiomyocytes, endothelial cells, vascular smooth muscle cells, fibroblasts, and the native cardiac stem cells. The overall effect is manifested by myocardial regeneration and neovascularization, culminating in reverse remodeling and better ventricular performance.

In our case, the maximum beneficial effect of cell-based therapy was 3 months after the cell delivery. This was also observed in our previously reported case of intramyocardial injection of autologous umbilical cord blood-derived mononuclear cells in a patient with HLHS.³¹ It is possible that the beneficial effects of cell-based therapy may be attenuated by unresolved structural and hemodynamic problems. In the present case, neo-aortic valve regurgitation posed a major hemodynamic concern. The patient was initially not a candidate for surgical valve replacement because of severe ventricular dysfunction. Improvement

TABLE 2. Summary of Cardiac Magnetic Resonance Imaging Data

| Magnetic resonance imaging parameters | Baseline | 6 mo |
|--|----------|-------|
| Right ventricular volumetric analysis | | |
| End-diastolic volume (mL) | 304 | 279 |
| Indexed end-diastolic volume (mL/m ²) | 213 | 192 |
| End-systolic volume (mL) | 200 | 173 |
| Stroke volume (mL) | 104 | 106 |
| Ejection fraction (%) | 34 | 38 |
| Myocardial deformation analysis (average values of all segments) | | |
| Four-chamber longitudinal strain (%) | -10.9 | -13.2 |
| Short-axis circumferential strain (%) | -10.3 | -13.4 |
| Short-axis radial strain (%) | 16.7 | 21.4 |

in his clinical status after medical management and cell-based therapy made him a better candidate for surgical intervention. Most patients with severe neo-aortic valve regurgitation have reduced ventricular function after valve replacement because of sudden reduction in preload, as was seen in our case. It is difficult to determine whether his rapid recovery of ventricular function can be attributed to his cell-based therapy 8 months earlier. Notwithstanding, cell-based therapy may prove to be an adjunct to medical and surgical therapy for the treatment of ventricular dysfunction. Severe neo-aortic valve regurgitation and resultant volume overload to the ventricle was likely the predominant cause of ventricular dysfunction in this case. It is possible that such individuals may have a better response to cell therapy similar to how they respond favorably to afterload reduction. Additional experience on a cohort of patients with varied physiology will be required to determine the optimal target populations and timing for these treatments.

The ventricular dysfunction in patients with single ventricle physiology is a difficult, multifactorial problem, attributed to factors such as prolonged hypoxia, multiple cardiopulmonary bypass runs, arrhythmia, increased pressure load for single RV, and increased volume load at earlier palliative stages and due to valve dysfunction. It often does not respond to standard heart failure management, other than temporary stabilization and symptomatic improvement. Progressive, medically refractory heart failure often leads to need for intravenous inotropic support and listing for cardiac transplant. Given the palliative nature of transplant and limited organ availability, it is essential to seek alternative treatment strategies to strengthen the native single ventricle. The experience with cell-based therapy in children with congenital heart disease is limited.³²⁻³⁷ To date, there has been only one published clinical trial in which autologous cardiosphere-derived cells were infused in the coronary arteries of children with HLHS after stage 2 and 3 palliative operations.^{38,39} This was a phase 1 clinical trial that showed that the delivery of such cells was safe and feasible. The 3-year follow-up revealed improved RV EF and somatic growth, with reduction in incident heart failure in children

receiving cell-based therapy compared with the control group. Further studies are needed addressing different clinical scenarios and cell types to establish the most safe, effective, and cost-effective cell-based therapy in congenital heart disease.

CONCLUSION

This is the first case report of intracoronary delivery of autologous BM-MNCs administered for ventricular dysfunction, 23 years after Fontan operation in an adult with HLHS and severe neo-aortic valve regurgitation. Several ventricular functional parameters and ventricular size improved, with maximum change noted 3 months after the cell delivery. Developing regenerative therapeutic options may help in delaying and preventing cardiac transplant in patients with such single ventricle physiology.

Abbreviations and Acronyms: **BM-MNCs** = bone marrow–derived mononuclear cells; **CK-MB** = creatine kinase-MB; **CMR** = cardiac magnetic resonance; **EF** = ejection fraction; **HLHS** = hypoplastic left heart syndrome; **NT-proBNP** = N-terminal pro-B-type natriuretic peptide; **RV** = right ventricle

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Correspondence: Address to Muhammad Y. Qureshi, MBBS, Division of Pediatric Cardiology, Mayo Clinic, 200 First St SW, Rochester, MN 55905 (Qureshi.Muhammad@mayo.edu).

Members of the Wanek Program Regenerative Medicine Pipeline include: Timothy J. Nelson, MD, PhD (program director); Karen M. Cavanaugh, CCRP (lead study coordinator); Jennifer M. Miller, MBA (senior program manager); Karen S. Miller, CCRP (study coordinator).

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