

A randomized study of autologous bone marrow–derived stem cells in pediatric cardiomyopathy

Sian Pincott PhD, MRCPCH, Deborah Ridout MSc, Margaret Brocklesby BSC, Angus McEwan FRCA, Vivek Muthurangu MD, MRCPCH, Michael Burch MD, FRCP, FRCPC

Background

Bone marrow mononuclear cell fraction has been used as therapy for dilated cardiomyopathy in adults. Although case series are reported, there are no randomized controlled studies in children.

Methods

We designed a randomized, crossover, controlled pilot study to determine safety and feasibility of intracoronary stem cell therapy in children. The primary safety end-point was freedom from death and transplantation or any complication that could be considered related to bone marrow injection or anesthesia (e.g., infection, malignancy, anaphylaxis, renal deterioration). Other end-points were magnetic resonance imaging measurements and N-terminal prohormone brain natriuretic peptide. Participants included 10 children (mean age 7.2 years; range, 2.2–14.1 years; 6 boys) with cardiomyopathy (New York Heart Association/Ross Classification II–IV). Patients were crossed over at 6 months.

Results

The original protocol was completed by 9 patients. The safety end-point was achieved in all. Ratio of the geometric means for treatment effect adjusting for baseline was assessed for end-diastolic and end-systolic volumes (EDV, ESV): 0.93 for EDV (95% confidence interval 0.88–0.99, $p = 0.01$), indicating EDV was on average 7% lower in patients after stem cell treatment, and 0.90 for ESV (95% confidence interval 0.82–1.00, $p = 0.05$), indicating ESV was on average 10% lower after stem cell treatment compared with placebo. The primary efficacy end-point ejection fraction was not met.

Conclusions

Bone marrow mononuclear cell therapy for cardiomyopathy is feasible and safe in children. Left ventricular volumes were significantly reduced 6 months

after stem cell injection compared with placebo, which may reflect reverse remodeling.

Keywords

pediatric dilated cardiomyopathy

autologous stem cells

heart failure

intracoronary injection

bone marrow

Stem cell treatment for heart failure has generated much interest. Although heart failure is much less common in children than in adults, the social and economic implications of care of children with heart failure are significant. Children frequently require hospital admissions¹ with a long duration of intensive care support, using costly surgical interventions such as the Berlin Heart device (Berlin Heart GmbH, Berlin, Germany) as part of the management of heart failure. The bone marrow mononuclear cell fraction has been used experimentally as therapy early after myocardial infarction in adults; it appears safe and has a modest effect on ventricular function.^{2, 3, 4} Studies in adults with dilated cardiomyopathy have also shown positive results.^{5, 6} Pediatric bone marrow is usually more cellular than bone marrow of adults, and an absence of co-morbidities may enhance the potential for repair and regeneration. As modest improvements are seen in adults after stem cell injections, one could anticipate similar injections in children to have the potential to be more effective.

A case series has been described of stem cell therapy in children with heart failure,⁷ but no randomized studies exist to our knowledge. Although the issue of stem cell therapy is often discussed in pediatrics, the lack of randomized studies has led to very few centers considering such therapy. Randomized controlled studies in pediatric heart failure are very difficult to facilitate, and very few have been undertaken, with the carvedilol trial by the Pediatric Carvedilol Study Group in North America being possibly the only completed such study.⁸ Stem cell studies are even more difficult because of the invasive nature of the therapy and, in contrast to adult studies, the need for general anesthesia in most cases.

To overcome the difficulties of recruiting suitable patient numbers to enable assessment of end-points such as ventricular volumes, we elected to use cardiac magnetic resonance imaging (MRI). Echocardiography, although

widely used for clinical evaluation, can have significant variability in pediatric cardiomyopathy assessment,⁹ but the accuracy and reproducibility of cardiac MRI allowed a considerable reduction in the patient numbers required to investigate ventricular volumes and ejection fraction (EF).¹⁰ Use of cardiac MRI permitted the successful completion of the first randomized controlled trial to our knowledge of the use of autologous bone marrow–derived stem cells in pediatric cardiomyopathy.

Methods

Study design

A randomized, crossover, controlled design was used for this pilot study. The Consolidated Standards of Reporting Trials (CONSORT) statement was used to guide the development of the protocol.¹¹ The study complies with the Declaration of Helsinki, the locally appointed ethics committee has approved the research protocol, and informed consent was obtained from the subjects (or their legally authorized representative). Ethical approval was obtained for this project in April 2008 (REC 08/H0713/37). The study is registered at ClinicalTrials.gov, and the ClinicalTrials.gov identifier is NCT02479776. A CONSORT flow diagram (Figure 1) was produced, and a project plan for a crossover study was designed. Children 1–16 years old at review who were either attending the Heart Failure Clinic at Great Ormond Street Hospital or referred for acute hospital admission and management of heart failure at Great Ormond Street Hospital were invited to participate in the study. The lower age limit was selected to permit coronary artery catheterization to be performed feasibly, taking into account the size of the patient. Inclusion and exclusion criteria are listed in Table 1.

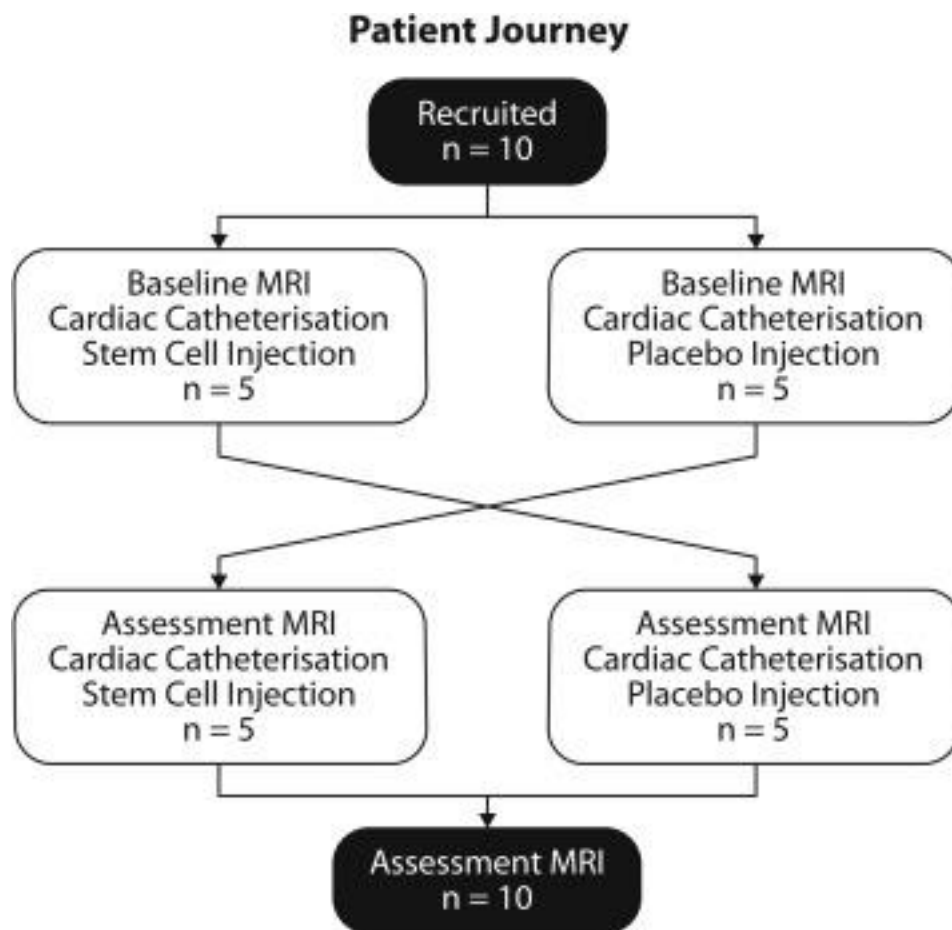


Figure 1. Patient journey illustrating the crossover study design.

Table 1. Inclusion and Exclusion Criteria

Inclusion criteria	Exclusion criteria
Pediatric cardiomyopathy dilated	Intravenous inotropes
Age 1–16 years	Ventilation (invasive and non-invasive)
Stable heart failure medication	High priority (urgent) listing for transplant
	Arrhythmia-induced cardiomyopathy
	Viral infections that would preclude use of hospital Cell Therapy Laboratory facilities
	Active malignancy
	Unstable oral drug therapy
	Age <1 year and age >16 years
	Weight <10 kg
	Major congenital heart disease with potential for hemodynamic effects on cardiac function ^a

a

Minor congenital heart disease was not excluded (e.g., patent foramen ovale/small atrial septal defect, silent ductus, bicuspid aortic valve with no stenosis or regurgitation).

Potential patients were identified from clinical case note review, and they and their families were approached either by telephone or following outpatient clinic review to evaluate their interest in participation. After preliminary briefing, any patients who appeared interested in participating in the study were given age-appropriate and language-appropriate written information; they were also given a contact telephone number for the research fellow (ESP) organizing the study so that additional information could be supplied if required. Each family was given the opportunity to discuss the study with the research team before consent for inclusion.

Protocol

There were 10 patients who met the eligibility criteria recruited for this study. Each patient was randomly assigned at entry to the study to determine whether the patient would receive stem cells at period 1 or period 2 of the crossover protocol (Figure 1). Randomization was performed using a computer-generated random number table (Graphpad; <http://www.graphpad.com/quickcalcs/RandMenu.cfm>).

Crossover of interventions occurred at 6 months with the stem cell group receiving placebo via the cardiac catheterization (no bone marrow harvest was required for the placebo arm of the study) on the second occasion. The same admission, monitoring, and follow-up were arranged as for the stem cell stage of the study.

After recruitment and randomization, patients had their individual admission timeline planned. Patients who were to receive stem cells at their first study admission were reviewed within the 28 days preceding that date. At this review, screening for infection, as per our hospital Cell Therapy Laboratory protocol (including human immunodeficiency virus and hepatitis B and C) was performed, following full written consent.

On the day of the procedure, the patient was admitted to the cardiac day-care facility for baseline observations and clinical assessment. The study procedure was performed in the cardiac MRI suite under general anesthetic. Blood samples were taken at the time of anesthesia for routine investigation (complete blood count, urea, electrolytes, liver function, and N-terminal prohormone brain natriuretic peptide [NT-proBNP]).

Bone marrow aspiration technique

The posterior iliac crest was used as the standard site for bone marrow aspiration. A volume of 20 ml of bone marrow was aspirated from a single needle puncture site using heparinized 10-ml syringes attached to the bone marrow needle following removal of the central trochar. The aspirated bone marrow was transferred to sterile heparinized universal specimen bottles. Immediately after aspiration, the bone marrow sample was taken to the on-site cell therapy laboratory for mononuclear cell separation using standard techniques of gradient centrifugation.

The mononuclear cell separation protocol took approximately 90 minutes to complete. The bone marrow sample was processed to provide 1 ml for intracoronary injection and 1 ml to be cryogenically frozen and stored. A small aliquot of stem cell suspension was analyzed to obtain total mononuclear cell counts per milliliter. This cell count represented the total number of mononuclear cells injected via intracoronary catheterization. Cell viability was also assessed. Further details of the cell preparation are given in the Supplementary Data (available in the online version of this article at www.jhltonline.org).

While waiting for the prepared stem cells, the cardiac MRI scan was performed using a breath-hold protocol for image acquisition. After the MRI scan, the patient was transferred via the mechanized sliding table back into the angiography suite.

On return to the angiography suite, the patient was maintained under general anesthesia until notice was given by the stem cell laboratory that the stem cell processing was nearing completion. Following contact with the stem cell laboratory, an arterial sheath was placed in the femoral artery of the patient and advanced until its tip was sited in the left main coronary artery. Partial occlusion of the coronary artery was observed as demonstrated by ST segment changes, and damping of the pressure trace was routinely monitored during such procedures. The 1-ml volume was slowly injected via the coronary artery catheter into the left main coronary artery followed by a 1-ml 0.9% saline flush over 2 minutes.

After intracoronary injection of stem cell suspension, the cardiac catheter was removed, and hemostasis at the skin entry site was achieved. Patients were discharged the following day and scheduled for elective review in the outpatient clinic at 3 months post-procedure and advised to contact the research fellow sooner if any concerns. The patients randomly allocated to placebo at stage 1 of the study crossed over to receive stem cells at stage 2 (Figure 1). Interim and final follow-up arrangements were the same for the stem cell and placebo groups.

Anesthesia was standardized for all patients and given by the same anesthetist; any adverse events were recorded in the operation notes. The anesthetist was part of the study group and was involved in pre-operative assessment discussions and immediate post-operative recovery. One patient in the study had severe Duchenne muscular dystrophy and was not considered suitable for general anesthesia. This patient completed the study under sedation. Bone marrow preparation was also standardized and performed by the same scientist in each case. Computer data entry was double-checked. Patient identifiers were removed from the anonymized images to allow blind analysis later on; in this way, unbiased image analysis was performed. A single researcher using OsiriX software performed off-line analysis of the data. As a second check of these data, the researcher's results were evaluated, again blind to patient identifiers, by a second analyzer. The key to the blinding of the MRI data was devised, applied, and stored securely by a clinician not otherwise involved in the study. Indexed values corrected the data for age, sex, height, and weight and permitted true comparisons to be made between the series of studies of the same individual and between different individuals.

The primary efficacy end-point was left ventricular ejection fraction (LVEF). We performed a power calculation using information in the literature to help with interpretation of the results. Statistical review of the available literature^{2, 3, 5, 12} indicated that a 3% change in LVEF as measured by MRI would represent a meaningful change. We calculated that 10 patients would be sufficient to detect a 3% mean change in EF, assuming a SD of difference of 3% with 80% power and 5% significance. For all calculations, it was assumed that the distribution of differences was normal. Calculation of a sample size for the crossover study required a definition of the minimum difference to be detected.

The primary safety outcome measures were freedom from death and transplantation or any complication that could be considered related to bone marrow injection or anesthesia (infection, malignancy, anaphylaxis, renal deterioration). We also collected data on unexpected hospitalization, emergency department visits that did not result in admission to the hospital, disability or permanent damage, any new persistent or permanent impairment or damage to the patient, need of implantation of a new medical device, serious blood dyscrasias (blood disorders), or seizures or convulsions that did not result in hospitalization. A safety monitoring committee, including a cardiologist from Great Ormond Street Hospital not involved in the trial, an external cardiologist, and a lay representative, was formed, and any adverse events were to be reported to the committee, which was empowered to halt the trial at any time if concerns arose. Safety events were collected at formal clinical assessment and interview with the patient and parents at the study visits and at 3 months after the procedure with

echocardiography and routine blood testing. Blood was taken for NT-proBNP at each visit. In addition, functional status was assessed by the New York Heart Association classification or the Ross Classification in younger children. Telephone contact with the study families was by the research fellow at approximately monthly intervals in between visits. The fellow made a note of any complications

All images were obtained with a 1.5-T MRI scanner (Avanto; Siemens, Erlangen, Germany) using a 12-element phased-array coil for signal reception and the body coil for signal transmission. A vector electrocardiography system was used for cardiac gating. Left ventricular volumes were measured from contiguous short-axis steady-state free precession cines covering both ventricles (7–13 slices, depending on the size of the child). Each slice was acquired in a single 10- to 15-second breath hold as previously described. In 1 patient with significant cardiac arrhythmia, a real-time radial k-space and time sensitivity encoding sequence was used because of difficulties in cardiac gating.

All image processing was performed using in-house plug-ins for the open-source OsiriX digital imaging and communications in medicine software. The left ventricular end-diastolic volume (EDV) and end-systolic volume (ESV) were measured by manual segmentation of the endocardial borders in the short-axis data. Careful segmentation of the basal slices in conjunction with 4-chamber and left ventricular long-axis views was performed to overcome problems with delineating the mitral valve. Ventricular stroke volume was the difference between the EDV and ESV, and ventricular EF (%) was $(\text{stroke volume}/\text{EDV}) \times 100$.

For EDV, ESV, and EF, comparisons between treatments were made on a per protocol basis. A crossover analysis on post-treatment measures was conducted using an analysis of variance model within the `pkcross` routine in Stata, considering sequence, period, and treatment effects. Carryover effect was tested first, and where there was no evidence of a significant effect, the treatment effect was investigated further. A subsequent generalized estimating equation model was fitted, which allowed for an adjustment for baseline measures. Estimates of treatment effects with 95% confidence intervals (CIs) using robust standard errors are presented.

Where the distribution of the data was clearly non-normal, we used a logarithmic transformation. Stata was used for all statistical analyses, and all tests were 2-sided. A p -value ≤ 0.05 was considered significant.

Results

From the clinic database, 40 patients were identified, and case notes were reviewed. Of these cases, 12 were unsuitable for the study, 15 were approached, 4 declined, and 11 agreed, but 1 patient had unstable heart failure that required inpatient care with inotropic therapy and did not enter the study. Mean age of the 10 study patients was 7.2 years (range, 2.2–14.1 years), 6 were boys, and all had cardiomyopathy (New York Heart Association/Ross Classification II–IV). The patients were recruited from the Heart Failure Clinic at Great Ormond Street Hospital. Patient profiles were obtained from the history taken either from the patient or from the family supported with data taken from individual case notes and investigation results. Clinical details are presented in Table 2.

Table 2. Patient Details

Patient	Age	Diagnosis	Time since heart failure presentation	NYHA/Ross Classification	SF in clinic	Medication
1	3 years, 11 months	Poor left ventricular function post-mitral valve replacement	2 years	II	16%	Captopril; carvedilol; warfarin; furosemide
2	6 years, 1 month	Anthracycline cardiomyopathy; acute myeloid leukemia	3 years	III	18%	Captopril; carvedilol; aspirin; furosemide
3	12 years, 8 months	Anthracycline cardiomyopathy; trisomy 21; acute myeloid leukemia	3 years	II	23%	Captopril; carvedilol; warfarin; furosemide; spironolactone; digoxin
4	14 years, 0 months	Anthracycline cardiomyopathy; mesoblastic nephroma (nephrectomy)	3 years	II	22%	Lisinopril; carvedilol; aspirin; furosemide
5	2 years, 2 months	Neonatal enterovirus myocarditis	3 years	III	23%	Captopril; carvedilol; warfarin; furosemide;

Patient	Age	Diagnosis	Time since heart failure presentation	NYHA/Ross Classification	SF in clinic	Medication
	months					spironolactone; digoxin
6	13 years, 6 months	Duchenne muscular dystrophy	1 year	II	15%	Perindopril; carvedilol
7	3 years, 9 months	Familial dilated cardiomyopathy, <i>MYH7</i> gene isolated	3 years	II	14%	Lisinopril; aspirin; carvedilol
8	2 years, 5 months	Idiopathic dilated cardiomyopathy, infant presentation	4 years	III	13.5%	Captopril; aspirin; furosemide; spironolactone; digoxin; carvedilol
9	9 years, 0 months	Neonatal enterovirus myocarditis	9 years	II	20%	Lisinopril; carvedilol; spironolactone
10	3 years, 9 months	Idiopathic dilated cardiomyopathy, infant presentation	4 years	II	19%	Captopril; carvedilol; aspirin; furosemide

NYHA, New York Heart Association; SF, shortening fraction.

Primary outcome measures

The primary safety end-point was achieved in all patients. There were no withdrawals from the study. There were no emergency department visits. There was no anaphylaxis. Blood results at the study visits showed stable hematology, liver function, and renal function. There were no seizures and no neurologic complications. No pacemakers or other new devices were needed during the study period.

The original protocol was completed by 9 patients; 1 patient had Duchenne muscular dystrophy, and general anesthesia was not undertaken because of the risk of malignant hyperthermia. The procedure was performed with the patient awake with local anesthesia without complications. As anesthesia is known to have cardiac effects, this change to local anesthesia was considered a significant deviation from the original study protocol, which resulted in the patient's data (patient reference number 6) being excluded from the subsequent analysis; there were no complications or safety issues with this patient. General anesthesia was well tolerated, and no inotropes were needed. Only transient ST segment changes were seen with engagement of the coronary artery, but this reversed after the catheter was withdrawn. No patients required vasodilators for coronary spasm. No patients required intensive care. All patients were monitored on the ward overnight and had electrocardiogram monitoring, but no arrhythmias were seen. No arrhythmias were reported during the follow-up period, although there was no per protocol monitoring such as Holter monitoring. Mononuclear cell counts are shown in Table 3; the mean, excluding patient 6 with Duchenne muscular dystrophy, was $54.4 \times 10^6/\text{ml}$ ($51.65 \times 10^6/\text{ml}$ including patient 6 with Duchenne muscular dystrophy).

Table 3. Cell Number Injected into Left Main Coronary Artery of Each Patient

Patient	Number of cells administered
1	115.1×10^6
2	34.1×10^6
3	11.8×10^6
4	31.5×10^6
5	34.2×10^6
6	26.9×10^6
7	64.5×10^6
8	73.5×10^6
9	35.5×10^6
10	88.8×10^6

Cell count = number of cells/ml from an aliquot of the suspension to be injected. Patient 6 was excluded from outcome analysis because of deviation from protocol.

The primary efficacy end-point for LVEF was not met. For EF and NT-proBNP, there was no evidence of a difference in treatments. The mean difference for EF, adjusted for baseline, was 1.73 (95% CI -2.01 to 5.46 , $p = 0.37$) and for NT-proBNP was 0.29 (95% CI -0.95 to 0.37 , $p = 0.39$). For

EDV, ESV, and EF, there was no evidence of carryover effect or period effect (Table 4). Indexed EDV 6 months after cell infusion (for 9 patients) was reduced in 6 patients and unchanged in 3 patients. EDV increased in 4 patients after placebo. The ratio of the geometric means for treatment effect, adjusting for baseline, was 0.93 (95% CI 0.88 to 0.99, $p = 0.01$), indicating EDV was on average 7% lower in patients after stem cell treatment compared with time after placebo. EDV was reduced in all patients with an injected cell count $>34.2 \times 10^6$. ESV decreased or stayed the same in 6 patients after cell injection. The ratio of geometric means for treatment effect, adjusting for baseline, was 0.90 (95% CI 0.82 to 1.00, $p = 0.05$), indicating ESV was on average 10% lower after stem cell treatment compared with placebo.

Table 4. Secondary Outcome Measures Before and After Stem Cell Intracoronary Injection^a

	Before stem cells	Before stem cells	Before stem cells	Before stem cells	After stem cells	After stem cells	After stem cells	After stem cells
	NT-proBNP (pg/ml)	EDV (ml)	ESV (ml)	EF (%)	NT-proBNP (pg/ml)	EDV (ml)	ESV (ml)	EF (%)
Mean	1,318	132.6	79.5	40	1,371	122.24 ^b	74.31 ^b	40
Range	180–6,285	81.33–283.78	43.69–165.59	33–49	22–4,055	77.73–275.73	41.76–173.41	46–53
	Before placebo	Before placebo	Before placebo	Before placebo	After placebo	After placebo	After placebo	After placebo
Mean	1,325	128.9	78.96	39	1,433	129.30	80.99	38
Range	99–4,055	76.48–275.73	41.76–173.41	31–48	180–4,340	79.71–254.06	45.53–166.62	29–45

NT-proBNP, N-terminal prohormone brain natriuretic peptide; EDV, end diastolic volume; EF, ejection fraction; ESV, end systolic volume.

a

Patient with Duchenne muscular dystrophy was excluded from statistical analyses.

b

Reached statistical significance.

Discussion

To our knowledge, this is the first randomized controlled study of autologous stem cells in pediatrics. The primary safety end-point, freedom from death and transplantation or any complication that could be considered related to bone marrow injection or anesthesia (e.g., infection, malignancy, anaphylaxis, renal deterioration), was achieved in all patients. We have shown that infusion of the cells was safe and feasible with a single anesthetic allowing time to harvest the marrow, prepare cells, perform MRI, and infuse the cells into the coronary arteries. The availability of cardiac MRI in the angiography suite enabled accurate assessment of ventricular volumes. The ratio of the geometric means for treatment effect adjusting for baseline was significantly different for both EDV and ESV. EDV was on average 7% lower and ESV was on average 10% lower after stem cell treatment compared with placebo. There appeared to be some relationship to the number of cells injected, with EDV reduced in all patients injected with a cell count $>34.2 \times 10^6$. The improvements in EF did not reach significance, although reversing remodeling and improving ventricular volumes may be more important in chronic heart failure.^{13, 14} In long-term follow-up of the SOLVD study, LVEF remained unchanged, but the improvement in volumes was thought to explain the impact of enalapril on outcome.¹⁴ Changes in LVEF are generally small in the published stem cell studies. Fisher et al¹⁵ previously showed in a systematic review and meta-analysis that bone marrow stem cell treatment improves LVEF with a mean difference of 2.62%. It is difficult to know whether the small changes in EF are clinically important, but meta-analysis has shown a significant reduction in the risk of mortality and rehospitalization caused by heart failure in association with small changes in EF.

Despite the relatively small marrow volumes harvested, the cell counts in this study are broadly similar to cell counts reported in adults. However, similar to many of the adult stem cell studies, we did not perform assessment of functional capacity of the cells. However, we had the advantage that cells were infused within 1 hour of harvest, which avoided cell loss from freezing and damage from storage.¹⁶ We obtained similar bone marrow–derived mononuclear cell counts from relatively small bone marrow harvests compared with adult studies. The mean cell count used in this protocol was 54.4×10^6 , obtained from a 20-ml aspirate of bone marrow. The total cell counts used in the adult studies with similar protocols ranged from 87×10^6 to 304×10^6 obtained from bone marrow harvests of 50–130 ml. Recent randomized studies in adult patients with dilated cardiomyopathy have quite conflicting results.^{17, 18} Although some of this variation may be related to harvesting methods, the adult bone marrow study populations are often heterogeneous with regard to co-morbidities such as age, smoking, and high cholesterol, all of which can influence stem cells.^{19, 20}

Although pediatric heart failure is uncommon, approximately 25% of affected children die or undergo transplantation within a year.^{1, 21} Modern technology with ventricular assist devices may help more very ill children survive, but with total donor numbers remaining constant, the waiting time for these patients will steadily increase.²² Pediatric cardiac units are under strain to cope with chronic heart failure. New therapies are urgently needed, but it is important not to leap into poorly assessed regimens. Stem cell studies are sometimes decried as “snake oil” treatments because of lack of proper randomized placebo-controlled studies. Expression of concern has been published recently regarding one publication.²³ Criticism is even harsher in pediatrics, where non-randomized treatments are often developed because invasive placebo limbs are so difficult. Even in non-invasive studies of pediatric heart failure, there is perhaps only a single high-quality randomized placebo-controlled drug study.⁸ Our study may help to reassure doubters that complex randomized placebo-controlled studies of stem cell therapy can be achieved safely in pediatric patients. Our data show a reduction in ventricular volumes, which is broadly comparable with adult data on autologous stem cells suggesting reversal of remodeling.⁴ However, it is important to be realistic; the modest improvements we report with stem cells do not demonstrate a panacea for pediatric dilated cardiomyopathy, but they are perhaps a starting point in the quest for effective cellular therapies for pediatric heart failure.

This study was designed as a crossover trial, and such studies are particularly suited to early stages in treatment development. The influence of confounding covariates is reduced because each crossover patient serves as his or her own control, and crossover designs are statistically efficient and thus require fewer subjects. The placebo used for the intracoronary injection used was 1 ml 0.9% saline, consistent with the placebo solution used in adult studies. The placebo limb did not include bone marrow aspiration. We considered that this would have no impact on the primary end-point of the safety of the active limb of the study. It was considered unethical to perform a placebo marrow aspiration if the cells were not going to be harvested and used. Furthermore, there is no indication from the literature that isolated marrow harvest has any cardiac impact unless it is aspirated through the sternum. It has been argued that granulocyte colony-stimulating factor priming will have an impact on stem cells, but as we did not use this in either limb, this was not relevant. We did include a placebo intracoronary injection, as coronary injection may have an effect on the myocardium, and this is in keeping with adult randomized studies.^{13, 14} This is a novel pilot study and is the first randomized controlled study to our knowledge of autologous stem cells in pediatric patients; therefore, safety was a major concern. Although a power calculation was performed for LVEF, we did not believe it would be reasonable to perform a power calculation on safety, given that we designed a small pilot study and planned to describe and report the findings.

An interim clinic review was scheduled at 3 months post-procedure, and a follow-up cardiac MRI scan was scheduled at 6 months. The time scale was chosen after review of the available adult literature, which showed that effects on the left ventricle were usually seen within 6 months. Adult stem cell studies with EF as the primary outcome measure tend to use 3 or 6 months post-injection. There is limited plasticity of bone marrow mononuclear cells to contribute to contractile recovery. Therefore, a single stem cell administration could not be expected to have a sustained effect on cardiac functional recovery, and encouraging results from repeated injections have recently been reported.²⁴ Most authors now consider that the potential beneficial effects of autologous bone marrow mononuclear cells are mediated by the release of paracrine factors and thus would not have long-term sustained effects. The literature suggests a peak effect of stem cells at 6 months.²⁵

Limitations and future directions

This was a small study, and the population was selected from the clinic; none of the children were hospitalized, and all were on stable oral medication. All patients had impaired systolic function on echocardiography at selection and were generally only mildly symptomatic. The diagnoses varied, which is common in a pediatric heart failure clinic, and as this reflects clinical practice, we considered it reasonable to include a heterogeneous population. Further studies may define the effectiveness in differing etiologies and in more severe heart failure.

There were no short-term major safety events using this protocol, but further study is required. Further studies are also needed to assess the impact of cell numbers, given the wide variation in the number of cells harvested and injected. The functional capacity of the cells has been assessed in some adult stem cell studies, and this too could be explored in future pediatric studies. This was a pilot study, and a larger efficacy trial in pediatric patients is needed, although the numbers may not need to be huge if MRI is used.¹⁰ In the future, the use of other sources of cells could be considered, such as mesenchymal stem cells, which have the advantage of being allogeneic and readily available.

Conclusions

This study shows that a randomized controlled study of bone marrow mononuclear cells is feasible and safe in children and opens the way for further such studies with this and other cell therapies. Left ventricular volumes were significantly reduced at 6 months after stem cell injection compared with placebo, which may reflect reverse remodeling.

Disclosure statement

None of the authors has a financial relationship with a commercial entity that has an interest in the subject of the presented manuscript or other conflicts of interest to disclose.

The Welton Foundation provided funding for this study.

References

R.E. Andrews, M.J. Fenton, D.A. Ridout, M. Burch

British Congenital Cardiac Association. New-onset heart failure due to heart muscle disease in childhood: a prospective study in the United Kingdom and Ireland

Circulation, 117 (2008), pp. 79-84

K.C. Wollert, G.P. Meyer, J. Lotz, *et al.*

Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomized controlled clinical trial

Lancet, 364 (2004), pp. 141-148

V. Schächinger, S. Erbs, A. Elsässer, *et al.*, REPAIR-AMI Investigators

Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction

N Engl J Med, 355 (2006), pp. 1210-1221

D.M. Clifford, S.A. Fisher, S.J. Brunskill, *et al.*

Stem cell treatment for acute myocardial infarction

Cochrane Database Syst Rev, 2 (2012), p. CD006536

U. Fischer-Rasokat, B. Assmus, F.H. Seeger, *et al.*

A pilot trial to assess potential effects of selective intracoronary bone marrow-derived progenitor cell infusion in patients with nonischemic dilated cardiomyopathy: final 1-year results of the transplantation of progenitor cells and functional regeneration enhancement pilot trial in patients with nonischemic dilated cardiomyopathy

Circ Heart Fail, 2 (2009), pp. 417-423

B. Vrtovec, G. Poglajen, L. Lezaic, *et al.*

Effects of intracoronary CD34+ stem cell transplantation in nonischemic dilated cardiomyopathy patients: 5-year follow-up

Circ Res, 112 (2013), pp. 165-173

S. Rupp, C. Jux, H. Bönig, *et al.*

Intracoronary bone marrow cell application for terminal heart failure in children

Cardiol Young, 22 (2012), pp. 558-563

R.E. Shaddy, M.M. Boucek, D.T. Hsu, *et al.*, Pediatric Carvedilol Study Group

Carvedilol for children and adolescents with heart failure: a randomized controlled trial

JAMA, 298 (2007), pp. 1171-1179

C.K. Lee, R. Margossian, L.A. Sleeper, *et al.*, Pediatric Heart Network Investigators

Variability of M-mode versus two-dimensional echocardiography measurements in children with dilated cardiomyopathy

Pediatr Cardiol, 35 (2014), pp. 658-667

N.G. Bellenger, L.C. Davies, J.M. Francis, A.J. Coats, D.J. Pennell

Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance

J Cardiovasc Magn Reson, 2 (2000), pp. 271-278

D. Moher, K.F. Schulz, D. Altman, CONSORT Group (Consolidated Standards of Reporting Trials)

The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials

JAMA, 285 (2001), pp. 1987-1991

K. Lunde, S. Aakhus

Intracoronary injection of mononuclear bone marrow cells after acute myocardial infarction: lessons from the ASTAMI trial

Eur Heart J Suppl, 10 (2008), pp. K35-K38

S. Brucks, W.C. Little, T. Chao, *et al.*

Contribution of left ventricular diastolic dysfunction to heart failure regardless of ejection fraction

Am J Cardiol, 95 (2005), pp. 603-606

B. Greenberg, M.A. Quinones, C. Koilpillai, *et al.*

Effects of long-term enalapril therapy on cardiac structure and function in patients with left ventricular dysfunction. Results of the SOLVD echocardiography substudy

Circulation, 91 (1995), pp. 2573-2581

S.A. Fisher, S.J. Brunskill, C. Doree, A. Mathur, D.P. Taggart, E. Martin-Rendon

Stem cell therapy for chronic ischaemic heart disease and congestive heart failure

Cochrane Database Syst Rev (4) (2014), p. CD007888

F.H. Seeger, T. Tonn, N. Krzossok, A.M. Zeiher, S. Dimmeler

Cell isolation procedures matter: a comparison of different isolation protocols of bone marrow mononuclear cells used for cell therapy in patients with acute myocardial infarction

Eur Heart J, 28 (2007), pp. 766-772

S. Hamshere, S. Arnous, T. Choudhury, *et al.*

Randomized trial of combination cytokine and adult autologous bone marrow progenitor cell administration in patients with non-ischaemic dilated cardiomyopathy: the REGENERATE-DCM clinical trial

Eur Heart J, 36 (2015), pp. 3061-3069

H. Martino, P. Brofman, O. Greco, *et al.*

Dilated Cardiomyopathy Arm of the MiHeart Study Investigators. Multicentre, randomized, double-blind trial of intracoronary autologous mononuclear bone marrow cell injection in non-ischaemic dilated cardiomyopathy (the dilated cardiomyopathy arm of the MiHeart study)

Eur Heart J, 36 (2015), pp. 2898-2904

A. Orlandi, E. Chavakis, F. Seeger, M. Tjwa, A.M. Zeiher, S. Dimmeler

Long-term diabetes impairs repopulation of hematopoietic progenitor cells and dysregulates the cytokine expression in the bone marrow microenvironment in mice

Basic Res Cardiol, 105 (2010), pp. 703-712

C. Schmidt-Lucke, S. Fichtlscherer, L. Rössig, U. Kämper, S. Dimmeler

Improvement of endothelial damage and regeneration indexes in patients with coronary artery disease after 4 weeks of statin therapy

Atherosclerosis, 211 (2010), pp. 249-254

P.M. Alexander, P.E. Daubeney, A.W. Nugent, *et al.*, National Australian Childhood Cardiomyopathy Study

Long-term outcomes of dilated cardiomyopathy diagnosed during childhood: results from a national population-based study of childhood cardiomyopathy

Circulation, 128 (2013), pp. 2039-2046

J. Cassidy, T. Dominguez, S. Haynes, *et al.*

A longer waiting game: bridging children to heart transplant with the Berlin Heart EXCOR device—the United Kingdom experience

J Heart Lung Transplant, 32 (2013), pp. 1101-1106

The Lancet Editors

Expression of concern: the SCIPIO trial

Lancet, 383 (2014), p. 1279

B. Assmus, S. Alakmeh, S. De Rosa, *et al.*

Improved outcome with repeated intracoronary injection of bone marrow-derived cells within a registry: rationale for the randomized outcome trial REPEAT

Eur Heart J, 37 (2016), pp. 1659-1666

J. Brickwedel, H. Gulbins, H. Reichenspurner

Long-term follow-up after autologous skeletal myoblast transplantation in ischaemic heart disease

Interact Cardiovasc Thorac Surg, 18 (2014), pp. 61-66

