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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)

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Aims Pre-clinical and few clinical studies suggest that transplantation of autologous bone marrow mononuclear cells (BMNC) improves heart function in dilated cardiomyopathies. Our objective was to determine if intracoronary injection of autologous BMNC improves the left ventricular ejection fraction (LVEF) of patients with non-ischaemic dilated cardiomyopathy (NIDCM).

Methods and results This study was a multicentre, randomized, double-blind, placebo controlled trial with a follow-up of 12 months. Patients with NIDCM and LVEF <35% were recruited at heart failure ambulatories in specialized hospitals around Brazil. One hundred and sixty subjects were randomized to intracoronary injection of BMNC or placebo (1:1). The primary endpoint was the difference in change of LVEF between BMNC and placebo groups as determined by echocardiography. One hundred and fifteen patients completed the study. Left ventricular ejection fraction decreased from 24.0% (21.6–26.3) to 19.9% (15.4–24.4) in the BMNC group and from 24.3% (22.1–26.5) to 22.1% (17.4–26.8) in the placebo group. There were no significant differences in changes between cell and placebo groups for left ventricular systolic and diastolic volumes and ejection fraction. Mortality rate was 20.37% in placebo and 21.31% in BMNC.

Conclusion Intracoronary injection of autologous BMNC does not improve left ventricular function in patients with NIDCM.

Clinical Trial Registration URL: http://www.clinicaltrials.gov. Unique identifier: NCT00333827.

Keywords Cardiomyopathy • Heart failure • Stem cells • Tissue therapy

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Introduction

The use of bone marrow-derived cells for therapy in non-ischaemic dilated cardiomyopathy (NIDCM) has been limited to few limited sample size trials, usually single-centre and not placebo controlled or double blind. The autologous bone marrow cells in dilated cardiomyopathy trial enrolled 44 patients, 24 of which were injected with bone marrow mononuclear cells (BMNC) in a non-blind study that reported a 5.4% increase in left ventricular ejection fraction (LVEF).[1] In TOPCARE-CMD, 33 patients were injected with BMNC and after 3 months the main finding was improved microvascular function with variable results in global and regional contractile function.[2] In 2011,Vrtovec *et al.*[3] reported the use of granulocyte colony-stimulating factor mobilized CD34+ cells in 28 patients randomized to cell therapy vs. 27 patients who served as controls. After 1 year follow-up, the patients receiving the cells showed significant improvements in LVEF, 6-min walk distance and in the combined secondary endpoint of mortality and heart transplantation. We previously reported that BCM injection in 24 dilated cardiomyopathy (DCM) patients was safe and improved peak oxygen consumption, six-min walk distance, and clinical conditions, although LVEF did not increase.[4] We now report the results of a multicentre, randomized, double-blind, and placebo controlled trial to test for the efficacy of intracoronary BMNC in patients with NIDCM.

Methods

Trial design, randomization, and masking

The trial was multicentre, randomized, double-blind, and placebo controlled. Patients were allocated to intracoronary treatment or placebo injection after block randomization (1:1 ratio). Cells in saline containing 5% autologous serum, or plain saline with 5% autologous serum were put in opaque10 mL syringes before being sent to the catheterization lab. Each center independently performed all interventions after training by the Coordinating Centre. A detailed description of the study design can be found in a previous publication.[5]

Inclusion and exclusion criteria

Patients meeting all of the following criteria were eligible for the study: previous diagnosis of heart failure according to Framingham criteria[6]; heart failure symptoms for at least 1 year, with aetiologic diagnosis of NIDCM according to World Health Organization[7] criteria; ages between 18 and 75; New York Heart Association (NYHA) functional class III or IV; appropriate drug therapy following the optimization period (4 weeks) and an echocardiogram showing ejection fraction $<35\%$.

Patients meeting any of the following criteria were ineligible for the study: valvular heart disease, except for functional mitral or tricuspid regurgitation; coronary angiography showing at least a 50% lumen obstruction in main coronary arteries; systemic hypertension, serologic diagnosis of Chagas disease; previously diagnosed sustained ventricular tachycardia episodes; alcohol or drug abuse; pregnancy; use of cardiotoxic drugs; serum creatinine >2.0 mg/dL or prior dialysis therapy; presence of implantable pacemaker or cardioverter defibrillator and any other co-morbidity that affects 2-year survival.

Participating centres, patient enrolment, and data collection

Eleven centres participated in this trial. Data were collected online through electronic case report forms (eCRFs) stored in two independent servers. Data collection were organized based in the sequential phases of the study and depended on completion of the previous eCRFs, without which the system was blocked.

Therapy optimization

Appropriate drug treatment for heart failure in DCM was defined as the use of the following drugs: digoxin (0.125–0.25 mg/day); spironolactone (25 mg/day); hydroclorothiazide (12.5–50 mg/day) and/or furosemide (minimum of 40 mg/day); enalapril (5 and 40 mg/day) or captopril (37.5–150 mg/day) or equivalent doses of angiotensin receptor blockers; carvedilol (6.25 and 50 mg/day) or equivalent dosage of other β-blockers.

All patients had their pharmacologic therapy optimized for at least 4 weeks before randomization and were maintained in this therapy throughout the study, unless doses had to be adjusted due to adverse effects.

Interventions (bone marrow aspiration, cell processing, and cell injection)

All patients were subjected to bone marrow aspiration. One hundred millilitres of bone marrow content were aspirated, under sedation and local anaesthesia, by iliac crest puncture. Mononuclear cells were isolated by Ficoll-Hystopaque gradient centrifugation (Amersham Pharmacia) as previously described.[20] Cell populations were characterized by flow cytometry, using antibodies to CD34, CD45, CD105, and CD133 (BD Biosciences). Two to 3 h after processing, a minimum of 10^8 cells, diluted in 20 mL of saline, were injected into all coronary arteries using an angioplasty catheter without balloon inflation. After catheterization patients stayed for 24 h in the ICU and for 72 h in the hospital ward. Serum creatine kinase MB (CK-MB) and troponin I levels were measured at 6 and 12 h after the catheterization procedure.

Outcomes

Baseline and follow-up evaluation consisted of clinical data, chest radiography, electrocardiogram (ECG), biochemistry and haematology, brain natriuretic peptide (BNP), echocardiogram, 6-min walk test, ergospirometry, and Holter monitoring. Clinical data included physical examination, blood pressure determination, Minnesota Living with Heart Failure Questionnaire[8] (MLHFQ), and NYHA Class. Electrocardiogram was performed as a 12-lead conventional recording. Biochemistry included electrolyte, urea, creatinine, glucose, aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, total bilirubin, and its fractions. Haematology included haematocrit, haemogram, and coagulation. Brain natriuretic peptide was determined by chemoluminescence using the TRIAGE device (Alere, Inc., San Diego, CA, USA; 2010). Echocardiography was performed according to the American Society of Echocardiography and European Association of Echocardiography recommendations[9] and LVEF was determined by Simpson's rule. A core laboratory validated the echocardiograms, based on random sampling of the recorded exams sent by the participating centres. Cell characterization by flow cytometry was performed by a core laboratory. Chest radiography, 6-min walk test, ergospirometry, and Holter monitoring were performed at baseline, 180 and 360 days post-procedure.

The primary endpoint was the difference, between BMNC and placebo groups, of the LVEF change from baseline at 6 and 12 months after treatment. Secondary endpoints evaluated changes in NYHA functional class, mortality rate, functional capacity (by 6-min walk test and maximum oxygen consumption by ergospirometry), and quality of life (MLHFQ).

Sample size and statistical methods

The study was designed to detect a 5% point difference between groups with 95% confidence and 80% statistical power, assuming an SD of 8 (see Supplementary material online, *File S2*).

In the efficacy analysis, patients were analysed in the group to which they were allocated. If the patient died or was subject to heart transplantation before an endpoint was measured, we imputed a fixed value for the variable that was worse than the worst value recorded for that variable. Because the imputation of values for deaths renders the distribution of the variables non-Gaussian, we used robust statistical methods based on 20% trimmed distributions in all numeric variables analyses. Categorical data are presented as count (proportion) and numeric data are presented as trimmed means; 95% confidence intervals were calculated for point estimates. Differences in numeric variables between groups were analysed with the Yuen-Welch test[10] (a robust analogue of the *t*-test). Differences in NYHA functional class were analysed with the Cochran-Armitage test for trend.[11] Statistical analyses were done with Stata13.1 (StataCorp LP, College Station, TX, USA; 2013), and we judged *P*-value of <0.05 to be significant.

Results

Enrolment and characteristics of patients

As shown in *Figure 1*, 356 patients were assessed for eligibility; 187 did not meet inclusion criteria and 8 declined to participate. As 1 patient died before randomization, 160 were enrolled and randomized, with 82 allocated to the cell and 78 to the placebo group. The two groups were well matched at baseline (*Table 1*). Patients were enrolled and randomized between January 2006 and December 2012, but 45 were not included in the efficacy analysis because they abandoned the study or withdrew consent. The remaining 115 patients, 61 in the BMNC group and 54 in the placebo group, were treated and followed up for the duration of the study (*Tables 2–7*).

Cell characterization

The trimmed mean number of mononuclear cells and the percentage of CD34+, CD133+, and CD105+ cells did not differ between treatment groups as shown in *Table 2*.

Outcomes

No serious adverse events that could be directly related to cell injection were recorded during the trial. CK-MB enzyme levels at 12 and 24 h after catheterization were 13.6 and 13.9 mg/dL for BMNC and 12.1 and 11.6 mg/dL for placebo group ($P = 0.547$ and 0.408, respectively). Continuous ECG monitoring during 48 h in the ICU did not detect an increase in life threatening arrhythmias.

Twenty-four patients died during follow-up, 13 in the BMNC group and 11 in the placebo. Three patients in the BMNC group were submitted to heart transplantation 6 months after the

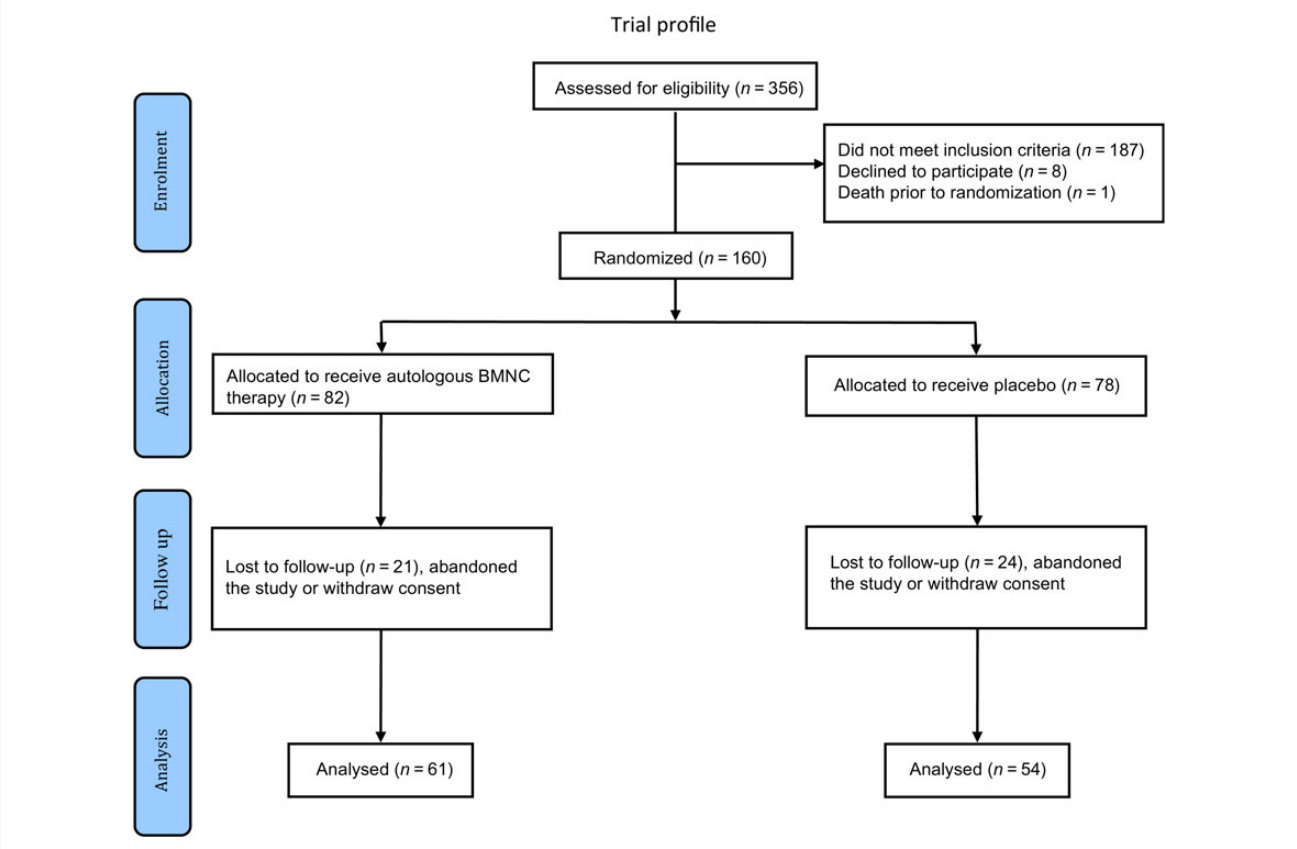

Figure 1 Study flow diagram showing the number of patients assessed, randomized and included in the final analysis. Bone marrow mononuclear cells indicate bone marrow-derived mononuclear cells.

Table 1 Baseline characteristics of the 160 patients randomized for the trial

	Placebo ($n = 78$)	BMNC ($n = 82$)
Age (years)	49.6 (11.1)	51.0 (11.1)
Men (%)	68.3	73.1
LVEF (%)	24.7 (7.0)	23.8 (7.2)
LVDV (mL)	258.2 (105.7)	251.1 (106.3)
LVSV (mL)	196.7 (91.7)	193.8 (89.5)
VO_2 max (mL/kg min)	15.2 (6.0)	15.2 (7.0)
Six-minute walk distance (m)	349.8 (139.7)	347.3 (146.7)
MLHFQ score	53.7 (19.4)	55.0 (21.1)
lnBNP (pg/mL)	5.4 (1.4)	5.7 (1.9)
ACEI (%)	61.1	64.1
ARB (%)	34.7	24.4
Hydralazine (%)	2.8	3.8
Nitrates (%)	5.6	3.8
Furosemide (%)	88.9	89.7
Spironolactone (%)	80.6	79.5
Hydroclorothiazide (%)	13.9	12.8
Digoxin (%)	79.2	76.9
Amiodarone (%)	9.7	12.8
Carvedilol (%)	86.1	83.3
Other β-blockers (%)	10.2	11

There were no statistical differences between groups for any of the variables analysed. Bone marrow mononuclear cells indicate bone marrow-derived mononuclear cells; LVEF, left ventricular ejection fraction; LVDV, left ventricular diastolic volume; LVSV, left ventricular systolic volume; MLHFQ, Minnesota Living with Heart Failure Questionnaire; lnBNP, natural logarithm of the brain natriuretic peptide concentration; ACEi, angiotensin converting enzyme inhibitors and ARB, angiotensin receptor blockers. Data are mean (SD) and for each drug the percentage of patients taking that drug is shown.

Table 2 Number of cells, viability, and CD 34, 105, and 133 content in the mononuclear fraction

	Placebo	BMNC
Total cells ($\times 10^8$)	2.64 (2.04–3.24)	2.36 (1.76–2.95)
Viability (%)	96.00 (94.99–97.01)	96.43 (95.55–97.32)
CD34 (%)	2.17 (1.80–2.54)	2.26 (1.80–2.73)
CD105 (%)	0.43 (0.32–0.53)	0.35 (0.24–0.45)
CD133 (%)	0.03 (0.02–0.04)	0.03 (0.02–0.04)

Values are trimmed means (95% confidence intervals). There are no significant differences between groups.

procedure. We therefore imputed 13 values for cell group at 6 months and 16 at 12 months. For the placebo group, we imputed 5 and 11 values at 6 and 12 months, respectively. All these patients were included in the analysis. Cause of death was pump failure for 9 patients (6 in cell and 3 in placebo group) and sudden cardiac death for 10 patients (6 in cell and 4 in placebo group). Five patients had non-cardiac deaths (2 in the cell and 3 in placebo group).

Mean LVEF in the BMNC group decreased at 6 and 12 months (*Table 3*). In the placebo group, LVEF increased at 6 months and decreased at 12 months (*Table 3*). Change in LVEF from baseline to 6 or 12 months did not differ significantly between treatment groups (*Table 3*).

Left ventricular diastolic and systolic volumes at baseline, 6 and 12 months follow-up are presented in *Table 4*. Left ventricular diastolic volume did not change significantly in the placebo group, but showed a significant increase at 12 months in cell-treated group. Left ventricular systolic volume decreased significantly at 6 and 12 months in both groups. The differences between groups on the changes in volume were not significant.

Maximal oxygen consumption decreased in BMNC and placebo groups. In fact, difference between groups was statistically significant at 6, but not at 12 months (*Table 5*). Six-minute walking distance did not vary significantly in both groups, and did not differ significantly between groups at both time points (*Table 6*).

Minnesota (MLHFQ) score significantly improved in placebo group at 6 months ($P = 0.013$) but difference between groups was not significant (*Table 7*). At 12 months both absolute values and difference to baseline between groups were not statistically different. Furthermore, change in NYHA functional class differed significantly between groups at 6 ($P = 0.003$) but not at 12 months ($P = 0.422$).

The number of hospital admissions did not differ significantly between groups at 6 ($P = 0.110$) or 12 ($P = 0.395$) months. Change in BNP concentrations also did not differ significantly between the groups ($P = 0.146$ and 0.241 at 6 and 12 months, respectively).

Discussion

Cell therapy for heart diseases using BMNC has yielded controversial results.

In ischaemic heart disease, clinical trials using BMNC have yielded variable results. Early reports of beneficial effects by Strauer[12] were followed by more robust trials.[13–16] In REPAIR-AMI, authors reported a significant gain in LVEF after BMNC therapy.[16] In a similar study, Lunde *et al.*[17] reported no significant gain in global EF after intracoronary BMNC injection. More recently, two clinical trials have also failed to detect global or regional LV function, in the TIME[18] or LateTIME[19] studies.

In non-ischaemic dilated cardiomyopathies, results using BMNC have also been variable. Seth *et al.*[1] reported an increase in EF by echocardiography in a single-centre, randomized, unblinded trial enrolling 44 patients, 24 of which received intracoronary injection of BMNC after coronary sinus balloon inflation. We published a safety and feasibility study with 24 patients with DCM, where although clinical evaluation showed improvement, EF did not increase as measured by echo and MRI.[4] Fischer-Rasokat *et al.* conducted an open label prospective study of 33 patients with DCM where BMNC were injected in the coronary arteries by the stop-flow technique.[2] They reported significant increases in LV wall motion and global EF after 3 months, attributing the beneficial effects of cell therapy to microvascular improvement.

We have found no benefit of BMNC therapy in mechanic (LVEF), anatomic (LV volumes), functional (VO_2 max and 6-min walk test), or clinical parameters (NYHA and MLHFQ) of patients with

Table 3 Left ventricular ejection fraction at baseline, 6 and 12 months for placebo and bone marrow mononuclear cell groups

	Placebo	BMNC	*P*
Baseline	24.3 (22.1 to 26.5)	24.0 (21.6 to 26.3)	0.830
6 months	25.2 (21.5 to 28.9)	22.9 (18.9 to 27.0)	0.394
Difference from baseline	0.9 (−2.1 to 4.0)	−1.1 (−5.3 to 3.2)	0.428
P	0.536	0.604	
12 months	22.1 (17.4 to 26.8)	19.9 (15.4 to 24.4)	0.469
Difference from baseline	−2.9 (−7.9 to 2.2)	−5.4 (−10.6 to −0.2)	0.469
P	0.257	0.044	

Differences from baseline to 6 and 12 months are also shown. *P*-values for differences between groups are shown in rightmost columns. *P*-values at left are differences from baseline to 6 and 12 months within groups. There are no significant differences in EF% between groups in any of the times analysed. Values are trimmed means (95% confidence intervals).

Table 4 Left ventricular diastolic and systolic volumes at baseline, 6 and 12 months for placebo and bone marrow mononuclear cell groups

	Placebo	BMNC	*P*
LVDV (mL)			
Baseline	254.0 (221.6 to 286.4)	240.6 (216.2 to 265.0)	0.496
6 months	219.2 (173.1 to 265.3)	274.0 (202.3 to 345.7)	0.179
Difference from baseline	−8.6 (−35.6 to 18.5)	15.3 (−12.2 to 42.8)	0.199
P	0.522	0.266	
12 months	299.1 (228.7 to 369.5)	308.6 (239.8 to 377.4)	0.389
Difference from baseline	58.9 (−5.9 to 123.7)	83.9 (19.9 to 148.0)	0.566
P	0.073	0.012	
LVSV (mL)			
Baseline	188.8 (160.9 to 216.6)	184.7 (161.2 to 208.3)	0.820
6 months	130.3 (99.1 to 161.4)	119.9 (86.7 to 153.1)	0.632
Difference from baseline	−27.9 (−48 to −7.8)	−51.7 (−88.7 to −14.6)	0.244
P	0.008	0.008	
12 months	116.0 (75.3 to 156.7)	95.3 (60.5 to 156.7)	0.416
Difference from baseline	−54.0 (−101.6 to −6.3)	−79.0 (−124.4 to −33.6)	0.427
P	0.028	0.001	

Differences from baseline to 6 and 12 months are also shown. *P*-values for differences between groups are shown in rightmost columns. *P*-values at left are differences from baseline to 6 and 12 months within groups. Values are trimmed means (95% confidence intervals).

Table 5 Maximal oxygen consumption (VO_2 max, mL/kg min) at baseline, 6 and 12 months for placebo and bone marrow mononuclear cell groups

	Placebo	BMNC	*P*
Baseline	14.6 (13.1 to 16.0)	14.9 (13.1 to 16.7)	0.775
6 months	15.4 (11.0 to 19.9)	9.9 (6.3 to 13.6)	0.052
Difference from baseline	−0.2 (−3.5 to 3.2)	−7.7 (−14.7 to −0.8)	0.047
P	0.927	0.03	
12 months	12.3 (7.2 to 17.4)	8.7 (4.6 to 12.9)	0.272
Difference from baseline	−4.0 (−9.1 to 1.1)	−8.4 (−13.3 to −3.5)	0.196
P	0.116	0.001	

Differences from baseline to 6 and 12 months are also shown. *P*-values for differences between groups are shown in rightmost columns. *P*-values at left are differences from baseline to 6 and 12 months within groups. Values are trimmed means (95% confidence intervals).

Table 6 Six-minute walk test at baseline (m), 6 and 12 months for placebo and cell groups

	Placebo	BMNC	*P*
Baseline	360.3 (328.1 to 392.5)	348.4 (312.4 to 384.4)	0.615
6 months	376.6 (326.4 to 426.7)	325.7 (233.2 to 418.2)	0.315
Difference from baseline	13.9 (−23.7 to 51.5)	−14.8 (−98.2 to 68.5)	0.514
P	0.455	0.719	
12 months	335.6 (238.4 to 432.9)	276.9 (176.4 to 379.2)	0.395
Difference from baseline	−18.0 (−87.3 to 51.4)	−47.5 (−118.5 to 23.5)	0.545
P	0.599	0.182	

Differences from baseline to 6 and 12 months are also shown. *P*-values for differences between groups are shown in rightmost columns. *P*-values at left are differences from baseline to 6 and 12 months within groups. There are no significant differences in LVDV between groups in any of the times analysed. Data are trimmed means (95% confidence intervals).

Table 7 Minnesota Living with Heart Failure Questionnaire at baseline, 6 and 12 months for placebo and cell groups

	Placebo	BMNC	*P*
Baseline	53.3 (47.0 to 59.6)	56.6 (50.3 to 62.8)	0.457
6 months	37.4 (28.6 to 46.2)	52.1 (38.2 to 65.9)	0.07
Difference from baseline	−13.3 (−23.6 to −3.1)	−2.4 (−15.2 to 10.3)	0.169
P	0.013	0.701	
12 months	41.6 (25.9 to 57.3)	59.1 (45.3 to 72.9)	0.086
Difference from baseline	−7.6 (−24.8 to 9.7)	5.3 (−9.7 to 20.2)	0.245
P	0.376	0.477	

Differences from baseline to 6 and 12 months are also shown. *P*-values for differences between groups are shown in rightmost columns. *P*-values at left are differences from baseline to 6 and 12 months within groups. There are no significant differences in LVDV between groups in any of the times analysed. Data are trimmed means (95% confidence intervals).

NIDCM. These results are compatible with the ones reported by the Chagas disease arm of the MiHearttrial.[20] In contrast to that trial, in the present study most of the measured variables deteriorated during the trial. In fact, there was a consistent trend across several variables for a better response in the placebo group. More specifically, with VO_2 max, there was a statistical significance at 6-month follow-up. This difference at 6 months may be entirely due to the imputation of the worst value of the series for patients who died before this variable was measured. In the BMNC group, 13 patients died during the 6 months follow-up, while in the placebo group only 5 patients died during this period. At 12 months, when five more patients died in the placebo group, the difference in maximal oxygen consumption between groups was no longer statistically significant.

Vrtovec *et al.*[3] reported that injection of $CD34^+$ cells in the coronary artery supplying segments with reduced viability in 28 patients with NIDCM led to significant improvements in global EF and in 1 year mortality when compared with the 27 patients in the control group. In a subsequent study enrolling 110 patients and followed for 5 years after intervention, Vrtovec *et al.*[21] injected 55 patients with $CD34^+$ cells. This single-blinded study showed a significant improvement in variables measured in the previous study, including overall mortality, in the cell group. An additional study comparing transendocardial and intracoronary injection routes demonstrated that direct intracardiac $CD34^+$ cell injection led to greater retention of cells and greater improvement in cardiac parameters.[22]

Altogether, Vrtovec's results contrast with the ones reported here, but the use of a true bone marrow stem cell population ($CD34^+$) renders comparisons difficult. As these trials were performed at a single centre, were not double-blind nor placebo controlled, and not powered to test for mortality, the significant differences in functional parameters and overall mortality suggest that $CD34^+$ cells should be more rigorously tested for DCM.

We conclude that autologous BMNC therapy, delivered by intracoronary injection, does not improve echocardiographic, functional or clinical parameters in patients with NIDCM.

Registration

This trial is registered with http://www.clinicaltrials.gov (unique identifier: NCT00333827).

Supplementary material

Supplementary Material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

Ethical approval

This study complies with the Declaration of Helsinki and was approved by the Brazilian National Ethics Commission. Informed consent was obtained from all subjects (or their guardians).

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