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# Direct intramyocardial transthoracic transplantation of bone marrow mononuclear cells for non-ischemic dilated cardiomyopathy: INTRACELL, a prospective randomized controlled trial

*Ensaio clínico prospectivo randomizado sobre terapia com células mononucleares da medula óssea para cardiomiopatia dilatada idiopática: INTRACELL*

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## Abstract

**Objective:** We tested the hypothesis that direct intramyocardial injection of bone marrow mononuclear cells in patients with non-ischemic dilated cardiomyopathy can improve left ventricular function and physical capacity.

**Methods:** Thirty non-ischemic dilated cardiomyopathy patients with left ventricular ejection fraction <35% were randomized at a 1:2 ratio into two groups, control and treated. The bone marrow mononuclear cells group received  $1.06 \pm 108$  bone marrow mononuclear cells through mini-thoracotomy. There was no intervention in the control group. Assessment was carried out through clinical evaluations as well as a 6-min walk test, nuclear magnetic resonance imaging and echocardiogram.

**Results:** The bone marrow mononuclear cells group showed a trend toward left ventricular ejection fraction improvement, with magnetic resonance imaging - at 3 months, showing an increase from  $27.80 \pm 6.86\%$  to  $30.13 \pm 9.06\%$  ( $P=0.08$ ) and returning to baseline at 9 months ( $28.78\%$ ,  $P=0.77$ ). Magnetic resonance imaging showed no changes in left ventricular ejection fraction during follow-up of the control group ( $28.00 \pm 4.32\%$ ,  $27.42 \pm 7.41\%$ , and  $29.57 \pm 4.50\%$ ). Echocardiogram showed left ventricular ejection fraction improved in the bone marrow mononuclear cells group at 3 months,  $25.09 \pm 3.98$  to  $30.94 \pm 9.16$  ( $P=0.01$ ), and one year,  $30.07 \pm 7.25\%$  ( $P=0.001$ ). The control group showed no change ( $26.1 \pm 4.4$  vs  $26.5 \pm 4.7$  and  $30.2 \pm 7.39\%$ ,  $P=0.25$  and  $0.10$ , respectively). Bone marrow mononuclear cells group

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Abbreviations, acronyms & symbols	
BMMC	Bone marrow mononuclear cells
DCM	Dilated cardiomyopathy
LVEF	Left ventricular ejection fraction
MLHF	Minnesota Living with Heart Failure
MRI	Magnetic resonance cardiac imaging
NIDCM	Non-ischemic dilated cardiomyopathy
NYHA	New York Heart Association
PO	Post-operatively

showed improvement in New York Heart Association functional class, from  $3.40 \pm 0.50$  to  $2.41 \pm 0.79$  ( $P=0.002$ ); patients in the control group showed no change ( $3.37 \pm 0.51$  to  $2.71 \pm 0.95$ ;  $P=0.17$ ). Six-minute walk test improved in the bone marrow mononuclear cells group ( $348.00 \pm 93.51$  m at baseline to  $370.41 \pm 91.56$  m at 12 months,  $P=0.66$ ) and there was a non-significant decline in the control group ( $361.25 \pm 90.78$  m to  $330.00 \pm 123.42$  m after 12 months,  $P=0.66$ ). Group comparisons were non-significant.

**Conclusion:** The trend of intragroup functional and subjective improvement was not confirmed when compared to the control group. Direct intramyocardial application of bone marrow mononuclear cells in non-ischemic dilated cardiomyopathy was not associated with significant changes in left ventricular function. Differences observed within the bone marrow mononuclear cells group could be due to placebo effect or low statistical power.

**Descriptors:** Cardiomyopathy, Dilated. Heart Failure. Cells. Myocardial Contraction. Transplantation.

#### Resumo

**Objetivo:** Testamos a hipótese de que a injeção intramiocárdica direta de células mononucleares de medula óssea em pacientes

portadores de cardiomiopatia dilatada não-iskêmica pode melhorar a função ventricular e a capacidade física.

**Métodos:** Trinta pacientes com cardiomiopatia dilatada não iskêmica e fração de ejeção 35% foram randomizados na razão 1:2 em grupos controle e tratado. Grupo células mononucleares de medula óssea recebeu  $1,06 \pm 108$  células mononucleares de medula óssea por mini-toracotomia. Grupo controle não recebeu intervenção. Avaliação foi feita clinicamente e por teste de caminhada 6 minutos (T6m), ressonância magnética e ecocardiogramas.

**Resultados:** Grupo células mononucleares de medula óssea mostrou tendência de melhora da Fração de ejeção - ressonância magnética aos 3 meses,  $27,80 \pm 6,86\%$  para  $30,13 \pm 9,06\%$  ( $P=0,08$ ), retornando ao basal aos 9 meses ( $28,78\%$ ,  $P=0,77$ ). Grupo controle não apresentou variação ( $28,00 \pm 4,32\%$ ;  $27,42 \pm 7,41\%$  e  $29,57 \pm 4,50\%$ ). Ecocardiogramas - fração de ejeção melhorou no grupo células mononucleares de medula óssea aos 3 meses:  $25,09 \pm 3,98$  para  $30,94 \pm 9,16$  ( $P=0,01$ ) e aos 12 meses ( $30,07 \pm 7,25\%$ ,  $P=0,001$ ), enquanto o controle não variou:  $26,1 \pm 4,4$  vs.  $26,5 \pm 4,7$  e  $30,2 \pm 7,39\%$ ,  $P=0,25$  e  $0,10$ , respectivamente). Células mononucleares de medula óssea melhorou classe funcional *New York Heart Association*:  $3,40 \pm 0,50$  para  $2,41 \pm 0,79$  ( $P=0,002$ ); controles não mudaram ( $3,37 \pm 0,51$  para  $2,71 \pm 0,95$ ;  $P=0,17$ ). T6m melhorou no grupo células mononucleares de medula óssea ( $348,00 \pm 93,51$  m inicial para  $370,41 \pm 91,56$  m aos 12 m,  $P=0,66$ ) e declinou sem significância no controle ( $361,25 \pm 90,78$  m para  $330,00 \pm 123,42$  m aos 12 meses,  $P=0,66$ ). Não houve diferenças significativas entre os grupos.

**Conclusão:** A tendência intragrupo de melhora funcional e subjetiva não se confirmou quando comparado com controle. Portanto, a injeção direta intramiocárdica de células mononucleares de medula óssea não se associou a mudança significativa na função ventricular. As diferenças observadas no grupo tratado poderiam ser devidas a efeito placebo ou a baixo poder estatístico.

**Descritores:** Cardiomiopatia dilatada. Insuficiência Cardíaca. Células. Contração Miocárdica. Transplante.

## INTRODUCTION

Dilated cardiomyopathy is a leading cause of heart failure and the primary indication for heart transplantation<sup>[1,2]</sup>. The prevalence of this disease tends to increase according to population ageing and survival improvement achieved through advances in pharmacological treatment and implantable devices<sup>[3]</sup>. However, current therapeutic approaches are palliative in the sense that they are unable to directly address the underlying problem of the loss of cardiac tissue<sup>[4]</sup>.

Cell therapy with bone marrow mononuclear cells (BMMC) has been associated with beneficial effects in patients with acute myocardial infarction and ischemic heart failure. Data for non-ischemic dilated cardiomyopathy (NIDCM), however, is more limited<sup>[5-7]</sup>. Experimental studies suggest that stem cells improve heart function through both paracrine regulation of cytokines production and cell transdifferentiation, albeit in a small proportion of the transplanted cells<sup>[8,9]</sup>. Two small trials, using BMMC through in-

tracoronary route, showed small, but significant increase in left ventricular ejection fraction (LVEF) after treatment<sup>[5,6]</sup>. Recently, intracoronary injection of CD34+ was associated with medium-term improvement of ventricular function, exercise tolerance, and long-term survival in randomized trial of patients with DCM<sup>[7]</sup>.

Intracoronary injection is associated with a small percentage of transplanted cells effectively retained in the myocardium, which could limit results when that route is used for cell therapy<sup>[10]</sup>. In a pilot study, we found that direct application of BMMC through a small thoracotomy was safe and associated with a significant, although transitory, increase in LVEF in patients with non-ischemic dilated cardiomyopathy (NIDCM)<sup>[11]</sup>. In the present study, we tested the hypothesis that direct intramyocardial injection of BMMC in patients with NIDCM could improve LVEF assessed by cardiac magnetic resonance imaging (MRI) and echocardiogram, physical capacity evaluated by the six-minute walk test (6WT), and heart failure class functional (NYHA), when compared to conventional treatment.

## METHODS

### Study design

We conducted an open, parallel-group, explanatory randomized study in a single center to evaluate the safety and efficacy of BMMC in patients NIDCM. Thirty patients that fulfilled the criteria for inclusion were randomly assigned to each of the groups by means of a computer software for simple randomization: BMMC or control, in a 2:1 proportion.

After randomization, functional status of both groups was assessed at baseline using (6WT), New York Heart Association (NYHA) and Minnesota Living with Heart Failure (MLHF) questionnaires, which had been previously validated for the Brazilian population<sup>[12]</sup>. We used cardiac magnetic resonance imaging (MRI) to measure left ventricular diameters and ejection fraction. Functional status was reassessed every three months and MRI repeated three and nine months after inclusion. All patients were treated by the same physician at a dedicated outpatient clinic, according to ACC/AHA Guidelines for the Diagnosis and Management of Heart Failure in Adults<sup>[1]</sup>. Functional status was evaluated by a separate team. In this article, we present the results of the first year of follow-up. We were unable to perform a blind study because of the invasive nature of the procedure proposed. The study was submitted to the National Commission in Research (CONEP) and approved. All patients signed an informed consent.

Patients in the BMMC group, in addition to conventional treatment, were submitted to BMMC transplantation as described below up to one month after randomization. In this group, we also conducted flow cytometry and immunohistochemistry to characterize bone marrow cells.

Our primary objective was to evaluate changes in left ventricular ejection fraction assessed with MRI. Secondary objectives consisted of evaluation of: 1) Safety; 2) New York Heart Association (NYHA) functional class and quality of life measured with Minnesota Living with Heart Failure Questionnaire; 3) Effects in mortality; 4) Left ventricular diameters using MRI.

### Inclusion and exclusion criteria

The study was approved by the Ethics Review Board of Instituto de Cardiologia do Rio Grande do Sul/ Fundação Universitária de Cardiologia (IC/FUC), as well as by the National Ethics Committee in Research of Brazil, under protocol number 10376 and registered in ClinicalTrials.gov under number: NCT00743639. Both the study and the registry were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from each patient.

We screened for patients with heart failure, LVEF less than 35% and functional class III or IV, despite full medical treatment. Specific inclusion criteria were: (1) Age between 20 and 65 years; (2) Diagnosis of non-ischemic cardiomy-

opathy for at least 12 months before enrollment; (3) Coronary angiography with normal coronary arteries, which defined cardiomyopathy as non-ischemic for the purposes of this trial; (4) negative serology for Chagas Disease; and (5) Absence of other significant systemic disease limiting mid-term prognosis. We excluded patients with: (1) Documented episodes of ventricular tachycardia; (2) Moderate to severe mitral regurgitation or any other significant valve disease; (3) History of myocardial infarction; and (4) Previous cardiac surgery.

### Bone marrow mononuclear cells isolation and transplantation

Approximately four hours before the operation and with the patient under sedation, a volume of about 80 mL of bone marrow was aspirated from the anterior iliac crest and placed in an anticoagulated medium. Mononuclear cell fraction was separated by density centrifugation over Ficoll-Hypaque-1077 (Sigma Diagnostics, St Louis, MO) and washed in a heparinized saline solution containing 5% autologous serum. Cells were counted and suspended in 5 mL saline solution for intramyocardial injection. A small fraction was utilized for sterility and viability tests and for immunophenotyping. Viability greater than 90% was considered acceptable.

For detection of surface antigens the cells were trypsinized, centrifuged, and incubated for 30 minutes at 4°C with phycoerythrin (PE)- or fluorescein isothiocyanate (FITC)-conjugated antibodies against human CD3, CD4, CD14, CD34, CD38 and CD45 (Pharmingen BD, San Diego, CA). Excess antibody was removed by washing. The cells were analyzed using a FACScalibur cytometer equipped with 488 nm argon laser (Becton Dickinson, San Diego, CA) with the CellQuest software. At least 10,000 events were collected. WinMDI 2.8 software was used for building histograms.

Surgical approach was through a left mini-thoracotomy, consisting of an approximately 5 cm incision in the anterolateral portion of the fifth left intercostal space to expose the pericardium. A T-shaped pericardial incision was made to access the free wall of the left ventricle. Coronary arteries were identified and the cell suspension was directly injected, using a 21F Butterfly needle that was introduced about 5mm intramyocardially and connected to an extension managed by the surgical assistant. Twenty 0.25 mL injections were given in the myocardium and in the anterior, lateral, posterior, and apical faces of the left ventricular free wall. After reviewing the hemostasis, pericardium was approximated, the thoracic cavity was drained and the chest wall closed.

### Echocardiogram

Two-dimensional echocardiograms were obtained for measuring end-systolic volume (ESV), end-diastolic volume (EDV), and LVEF, according to standard protocols. All exams were performed by the same person, who was unaware of the

treatment group and was not part of the study team. It should be noted, however, that treated patients had a visible scar in the left chest, which made it impossible to guarantee blindness to the echo examiner.

### Magnetic Resonance Imaging

All MRI image analyses were performed by the same investigator, which was blind to the assignment of the patient. We used a 1.5-T scanner (GE Excite HDx) with ECG gating and a 4-element phased-array surface coil. Scan planes were planned according to standard procedures. Endocardial and epicardial borders were traced in all end-diastolic and end-systolic short-axis slices to determine LV end-diastolic and end-systolic volumes (LVEDV and LVESV). Global LVEF was calculated  $[(LVEDV-LVESV)/LVEDV]/100$  LVEDV. Systolic ventricular wall thickening was calculated for different heart segments and it was used for an already published sub-study<sup>[11]</sup>.

### Statistical analysis

Continuous variables are presented as mean $\pm$ SD, if not stated otherwise. Categorical variables were compared with the  $\chi^2$  test or Fisher's exact test. Statistical comparisons between initial and follow-up data were performed in a nonparametric paired fashion using the Wilcoxon signed rank test. Nonparametric Mann-Whitney U and Kruskal-Wallis tests were used to compare continuous and categorical variable, as well as the results between different groups. All tests were performed as 2-sided tests at a significance level of 0.05.

In the first three months of follow-up, 25% of the patients from the BMMC group had either died or been withdrawn from the study, resulting in a decrease in the number of treated cases available for late follow-up. Since those patients had a lower mean ejection fraction than the whole group (18.26 % vs. 21.75%), we excluded them from comparative analysis, in order to avoid overestimation of treatment effect. In other words, outcome analysis was performed in as-treated basis, not as intention-to-treat.

## RESULTS

### Baseline

Baseline characteristics are detailed in Table 1. There were no significant differences in baseline data between BMMC and control groups. All patients had severe heart failure and were highly symptomatic by the time of inclusion, despite maximum medical therapy.

### Cell analysis

A mean of  $1.06\pm0.43 \times 10^8$  mononuclear cells per patient were available for injection using this method. Cell viability was greater than 99%, and fungal and bacterial cultures were negative.

Cells from the first five samples were analyzed in greater detail. Immunophenotyping of the mononuclear cell fraction showed the following composition: CD34+ cells (1.5 $\pm$ 0.7%), CD45+ (74.6 $\pm$ 8.5%), CD14+ (8.4 $\pm$ 4.7%), CD3+CD4+ (22.8 $\pm$ 4.6%), CD3+CD8+ (8.2 $\pm$ 6.1%), and CD34+CD38-cells (0.7 $\pm$ 0.5%).

### Procedural safety and clinical outcomes

Surgical procedure was effectively performed in 19 patients, since one patient asked to be withdrawn soon after randomization. Table 2 summarizes surgical results. Four subjects died in the first month after the procedure. Two died of refractory cardiogenic shock in the first 72 hours post-operatively (PO). One of them presented with cardiac tamponade 7 hours after surgery and was submitted to re-operation. The patient's hemodynamic condition continued to deteriorate and he died due to cardiogenic shock 3 days PO. Another patient died on the 15<sup>th</sup> day PO due to incessant ventricular tachycardia. Finally, one patient died on the 28<sup>th</sup> day PO: he had been discharged from hospital, but was readmitted one week later due to heart failure, which was refractory to treatment. All of the patients above had LVEF below 21% before operation.

Table 1. Baseline patients characteristics.

	BMMC group (n=20)	Control group (n=10)
Male	13 (65%)	5 (50%)
Age	48.3 (8.71)	51.6 (7.79)
NYHA class	3.5 (0.52)	3.35 (0.48)
Minnesota QOL score (Minnesota)	66.2 (22.46)	50.6 (14.84)
Six minute walk test (min)	358.5 (88.69)	353 (86.67)
LVEF by ECHO (%)	21.75 (41.19)	24.76 (4.64)
Final diastolic diameter, ECHO (mm)	73.4 (8.18)	70.3 (7.4)
LVEF by MRI (%)	27.80 $\pm$ 6.86	28.00 $\pm$ 4.32
Nº of injected cells	$1.06 \times 10^8$	none

Data expressed as mean (% or SD);

LVEF by ECHO=left ventricular ejection fraction by echocardiogram; MRI=nuclear magnetic resonance imaging; QOL=quality of life by Minnesota Living with Heart Failure Questionnaire. There were no statistically significant differences between baseline groups characteristics

Table 2. Surgical results.

Surgery	Mean $\pm$ DP	Max value
Thoracotomy procedure (min)	84.7 $\pm$ 29	120
Post-operative, %		
Length of ICU Stay (days)	1.86 $\pm$ 1.3	6
Length of hospital stay (days)	4.50 $\pm$ 2.0	9



Three patients from the BMMC group died between 30 days and 12 months after surgery. In two patients, death was sudden, at 10 and 11 months PO. Another patient died 6 months PO due to heart failure.

In the control group, one patient died three months after randomization due to heart failure and another patient was lost for follow-up two months after randomization. There was no statistically significant difference in safety outcomes between BMMC and control groups: Fischer's test showed no difference in mortality between groups in the first 30 days ( $P=0.371$ ) or up to 12 months ( $P=1.000$ ).

### Effects of BMMC on LV Function

#### MRI evaluations

Patients of the BMMC group maintained LVEF at 3 months, from  $27.80\pm 6.86\%$  to  $30.13\pm 9.06\%$  ( $P=0.08$ ), and at 9 months ( $28.78\%$ ,  $P=0.77$ ). The control group also maintained systolic function during follow-up ( $28\pm 4.32\%$  vs.  $27.4\pm 7.4\%$ , at 3 months,  $P=0.79$ , and vs.  $29.57\pm 4.50$  at 9 months,  $P=0.46$ , respectively). Differences between groups were non-significant.

Both groups maintained end-systolic and end-diastolic volumes during follow-up. (Table 3).

#### Echocardiographic evaluations

Patients of the BMMC group had a significant improvement in left ventricular ejection fraction 3 months after the pro-

cedure, from  $25.09\pm 3.98$  to  $30.94\pm 9.16$  ( $P=0.01$ ). This benefit was maintained after one year of follow-up ( $30.07\pm 7.25$ ,  $P=0.001$ ). The control group has showed no change in the same period ( $26.1\pm 4.4$  vs.  $26.5\pm 4.7$  and  $30.2\pm 7.39$ ,  $P=0.25$  and  $0.10$ , respectively). Differences between treated and control groups, however, were not statistically significant. Left ventricular fractional shortening evaluation showed similar results. End-systolic and end-diastolic diameters had a small reduction in both groups. (Figure 1, Table 4).

### Quality of life and 6-minute walk test

Quality of life, evaluated by MLHFQ (Minnesota Living with Heart Failure Questionnaire), improved significantly in the BMMC group three months after the procedure ( $69.00\pm 20.71$  vs.  $43.13\pm 27.92$  points,  $P=0.005$ ), a benefit maintained during 6 and 12 months of follow-up ( $48.92\pm 24.26$  points,  $P=0.018$ ;  $37.08\pm 21.15$  points,  $P=0.001$ ; respectively). Patients of the control group showed a non-significant improvement, with smaller differences between baseline and 12 months follow-up (minus 31.92 points for BMMC group and minus 7.16 points for Control group). Differences between groups were not statistically significant. Functional class, evaluated using NYHA Classification, showed similar results with a statistically significant improvement in the BMMC group, no change in the control group. However, once again, there were no statistically significant differences between groups. (Figure 2, Table 5).

Table 3. Left ventricular volumes and function as assessed by Magnetic Resonance Imaging (MRI).

Parameter	BMMC group (n = 15)	Control group (n=9)	P
Ejection Fraction, %			
Baseline	$27.80\pm 6.86$	$28.00\pm 4.32$	NS
3 months	$30.13\pm 9.06$	$27.42\pm 7.41$	NS
Change from baseline	2.33	-0.58	
P value comparing to baseline	0.08	0.79	
9 months	$28.78\pm 9.97$	$29.57\pm 4.50$	NS
Change from baseline	0.98	+1.57	
P value comparing to baseline	0.77	0.46	
End-diastolic volume, mL			
Baseline	$272.73\pm 63.41$	$250.85\pm 67.00$	NS
3 months	$264.86\pm 72.24$	$222.42\pm 96.19$	NS
Change from baseline	-7.87	-28.43	
P value comparing to baseline	0.41	0.32	
9 months	$258.64\pm 82.97$	$258.85\pm 75.55$	NS
Change from baseline	-14.09	+8.00	
P value comparing to baseline	0.54	0.71	
End-systolic volume, mL			
Baseline	$200.40\pm 61.59$	$182.00\pm 55.70$	NS
3 months	$195.93\pm 78.49$	$161.67\pm 67.83$	NS
Change from baseline	-4.47	-20.43	
P value comparing to baseline	0.66	0.18	
9 months	$190.00\pm 82.18$	$184.14\pm 58.55$	NS
Change from baseline	-10.40	2.14	
P value comparing to baseline	0.73	0.91	

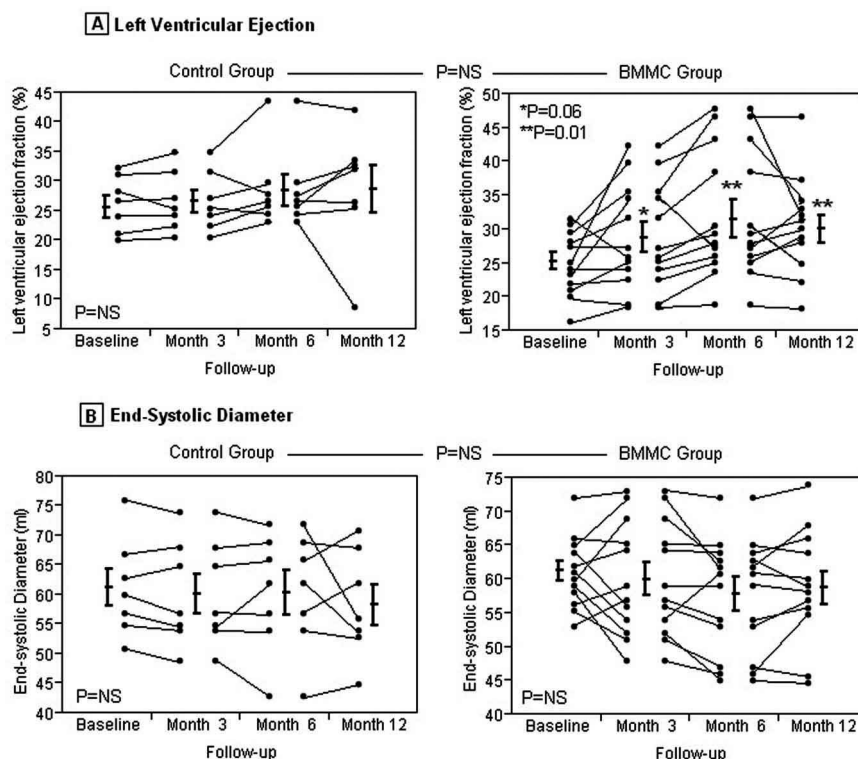


Fig.1 - A) Left ventricular ejection fraction and B) end-systolic diameter by echocardiography at baseline and during follow-up

Six-minute-walk test results were maintained in the BMMC group (from  $348.00 \pm 93.51$  min in baseline to  $370.41 \pm 91.56$  min after 12 months,  $P=0.66$ ) and in control group (from  $361.25 \pm 90.78$  min in baseline to  $330.00 \pm 123.42$  min after 12 months,  $P=0.66$ ). Again, differences between groups were not statistically significant. (Table 5).

## DISCUSSION

In this study, we tested the hypothesis that transplantation of BMMC through a small thoracotomy could improve left ventricular function in patients with refractory heart failure due to NIDCM. Our main findings were: (1) Mortality associated with the procedure was directly related to preoperative disease severity. In particular, patients with LVEF below 21% had a poor surgical result. Early deaths were related to post-operative pump-failure and congestive heart failure, while deaths at medium term were sudden, probably caused by cardiac arrhythmias. These results differ from our previous pilot study, in which there had been no deaths<sup>[11]</sup>. (2) Survivors treated with BMMC maintained left ventricular function relative to baseline according to MRI. (3) Overall, patients had symptomatic improvement during follow-up, which was

more pronounced in the BMMC group, but there was no statistical difference between groups in the outcomes studied.

The seven deaths (35%) observed in patients who received BMMC were due to pump-failure in the early post-operative period (2 cases; 28.5%), congestive heart failure (2 cases, 28.5%) and documented ventricular arrhythmia or sudden death (3 cases, 42.8%). Using the Seattle Heart Failure Model, a tool with excellent accuracy for predicting survival among patients with heart failure<sup>[13]</sup>, we could anticipate one-year mortality between 20% and 25%. Incidence of death attributable to arrhythmia (42.8%) was higher than expected for patients in NYHA functional class III (10.5%) or IV (18.6%) at one year<sup>[14]</sup>.

This higher than expected risk of ventricular tachyarrhythmia after surgery could be related to the intrinsic high risk of any procedure in patients with severe heart failure or to a more specific mechanism secondary to BMMC injection. Stem cell pro-arrhythmic potential is a matter of intense debate<sup>[15,16]</sup>. Clinically, bone marrow progenitor cells were not associated with increased risk of arrhythmias using different routes of delivery in different clinical scenarios<sup>[17-19]</sup>. Yet, experimental studies have shown that mesenchymal stem cells (MSCs) implantation altered cardiac conduction<sup>[20]</sup> and created areas of slow conduction, predisposing it to reentry

Table 4. Left ventricular volumes and function as assessed by echocardiography (ECO).

Parameter	BMMC group (n = 15)	Control group (n=9)	P
Ejection Fraction, %			
Baseline	25.09±3.98	26.10 ± 4.49	NS
3 months	28.11±7.22	26.525 ± 4.71	NS
Change from baseline	3.02	0.425	
P value comparing to baseline	0.06	0.628	
6 months	30.94±9.16	28.51 ± 6.98	NS
Change from baseline	5.85	2.41	
P value comparing to baseline	0.01	0.250	
12 months	30.07±7.25	30.23±7.39	NS
Change from baseline	4.98	4.13	
P value comparing to baseline	0.01	0.100	
End-diastolic diameter, mm			
Baseline	69.80±4.41	69.375±7.81	NS
3 months	68.33±6.38	68.62±8.39	NS
Change from baseline	-1.47	0.75	
P value comparing to baseline	0.18	0.170	
6 months	68.28±6.48	69.85±9.66	NS
Change from baseline	-1.52	0.479	
P value comparing to baseline	0.27	0.98	
12 months	68.66±7.35	68.28±8.47	NS
Change from baseline	-1.14	-1.09	
P value comparing to baseline	0.49	0.612	
End-systolic diameter, mm			
Baseline	61.46±4.65	60.75±7.86	NS
3 months	60.33±7.64	60.00±8.28	NS
Change from baseline	-1.13	-0.75	
P value comparing to baseline	0.47	0.28	
6 months	58.14±8.12	60.42±9.98	NS
Change from baseline	-3.32	-0.33	
P value comparing to baseline	0.08	0.63	
12 months	58.83±8.50	58.42±9.10	NS
Change from baseline	-2.63	-2.33	
P value comparing to baseline	0.17	0.42	

episodes<sup>[21,22]</sup>. Intramyocardial injections can create clusters of cells leading to heterogeneity in conduction and have been associated with greater risk of ventricular premature complexes than intracoronary injection<sup>[23]</sup>. How this translates into clinical practice, where patients are treated with anti-arrhythmic drugs, needs to be further clarified. At this point, however, we cannot dismiss the possibility of a pro-arrhythmic effect related the newly implanted cells.

BMMC treated patients who survived for more than one month after the procedure showed significant improvements in both symptoms and quality of life. Left ventricular ejection fraction was maintained one year after the procedure ( $P=0.77$ ). Results with echocardiography are more pronounced as they showed improvement in LVEF of patients treated with BMMC after 6 months (from  $25.09\pm3.98$  to  $30.94\pm9.16$ ,  $P=0.01$ ). Nevertheless, since operators were not blind for patients' group, results from this method should be interpreted with caution.

Clinical experience with mononuclear cells in patients with DCM is limited. Fischer-Rasokat et al performed infusion of BMMC in 33 patients with DCM using an over-the-wire balloon catheter in the left descending coronary artery<sup>[8]</sup>. After 3 months, regional wall motion of the target area and global left ventricular ejection fraction had a small (about 10%) but statistically significant improvement. Increase of regional contractile function was directly related to the functionality of infused cells as measured by their colony-forming capacity. Safety data was remarkably good, with no procedure related complications and no deaths, stroke or myocardial infarction up to one year follow-up. Compared to our study, patients were less symptomatic (67% in NYHA class II) and had better ventricular function (mean LEVF=0.2%).

Vrtovec et al.<sup>[7]</sup> conducted a study with 110 patients with NIDCM, which were randomized to receive CD34+ stem cell transplantation and 55 received no cell therapy. Patients underwent myocardial scintigraphy and cells were injected



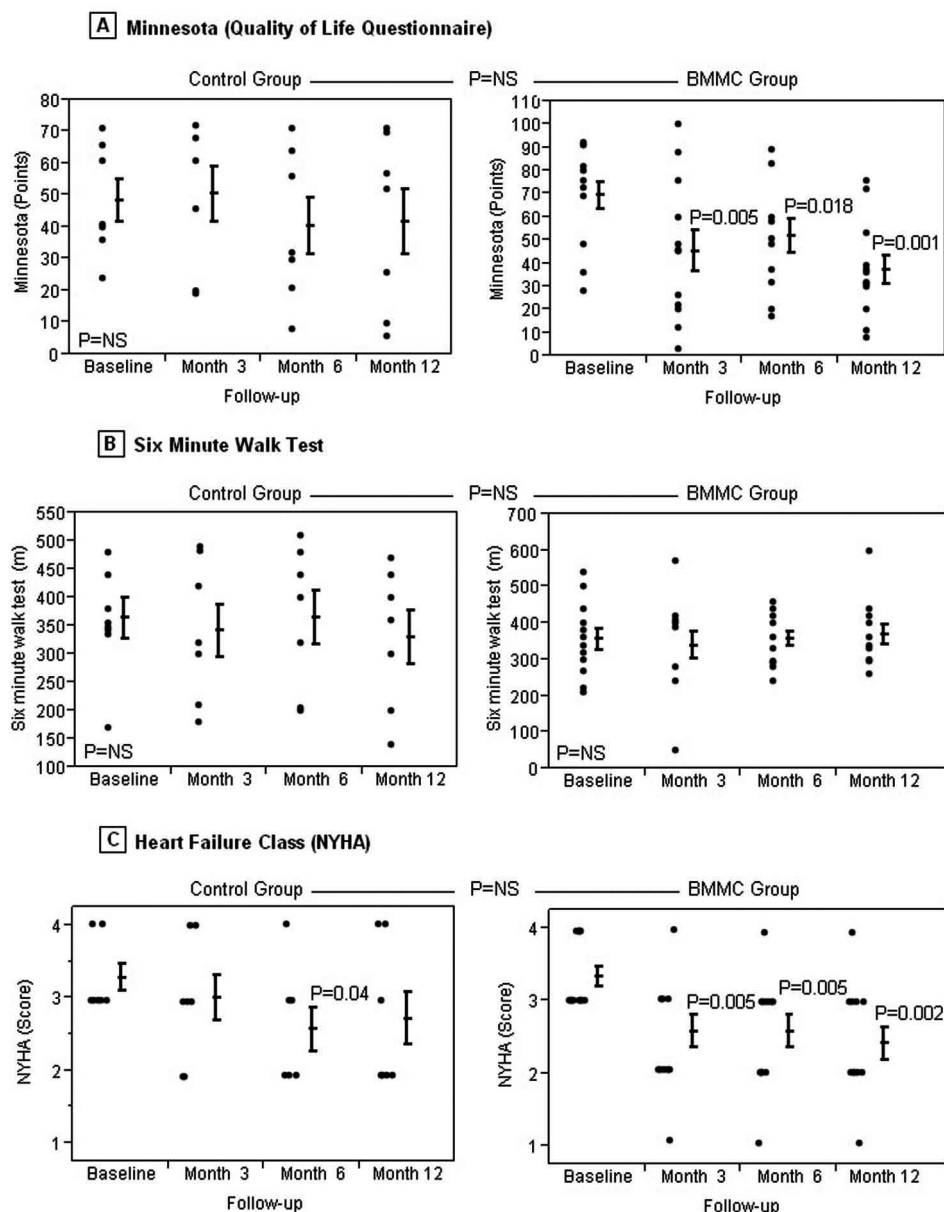


Fig. 2 - A) Quality-of-Life score (Minnesota), B) Six-minute walk test and C) NYHA Functional class at baseline and during follow-up

in the artery supplying segments with the greatest perfusion defect. At 5 years, stem cell therapy was associated with increased LVEF (from  $24.3 \pm 6.5\%$  to  $30.0 \pm 5.1\%$ ;  $P=0.02$ ), increased 6-minute walk distance (from  $344 \pm 90$  m to  $477 \pm 30$  m;  $P<0.001$ ), and decreased N-terminal B-type natriuretic peptide (from  $2322 \pm 1234$  pg/mL to  $1011 \pm 893$  pg/mL;  $P<0.01$ ). Left ventricular ejection fraction improvement was more significant in patients with higher myocardial homing of injected cells. Total mortality was lower in patients treated with CD 34+ cells (14% vs. 35%;  $P=0.01$ ) and the procedure was considered safe, with low morbimortality.

Several limitations of our study are recognized. First: the trial was projected with a small sample that was further reduced due to early mortality. This drawback was unexpected, since we had no early major complications or mortality in a previous safety study<sup>[11]</sup>. High mortality could be attributed to very low LVEF, which expressed extremely deteriorated LV function in those patients who died early. There was no low LVEF limitation for patient inclusion in the trial: all patients with  $LVEF<35\%$  were candidates. Perhaps we could have avoided including patients with very low LVEF (for example,  $LVEF<25\%$ ) or, alternatively, once they were included, we

Table 5. Minnesota Living with Heart Failure Questionnaire, New York Heart Association Functional Class and 6-Minutes-Walk Test results.

Parameter	BMMC group (n = 15)	Control group (n=9)	P
Minnesota LHFQ			
Baseline	69.00±20.71	48.87±16.32	NS
3 months	43.13±27.92	54.37±23.71	NS
Change from baseline	-25.87	+5.50	
P value comparing to baseline	0.005	0.57	
6 months	48.92±24.26	40.28±23.59	NS
Change from baseline	-20.08	-8.58	
P value comparing to baseline	0.018	0.31	
12 months	37.08±21.15	41.71±27.46	NS
Change from baseline	-31.92	-7.16	
P value comparing to baseline	0.001	0.48	
6-minutes-walk test			
Baseline	348.00±93.51	361.25±90.78	NS
3 months	338.66±116.48	336.25±114.38	NS
Change from baseline	-9.34	- 25	
P value comparing to baseline	0.73	0.44	
6 months	355.35±67.77	365.00±126.45	NS
Change from baseline	7.35	3.75	
P value comparing to baseline	0.81	0.98	
12 months	370.41±91.56	330.00±123.42	NS
Change from baseline	22.41	-31.25	
P value comparing to baseline	0.66	0.39	
NYHA			
Baseline	3.40±0.50	3.37±0.51	NS
3 months	2.60±0.82	3.00±0.75	NS
Change from baseline	-0.8	- 0.37	
P value comparing to baseline	0.005	0.19	
6 months	2.50±0.75	2.57±0.78	NS
Change from baseline	-0.9	-0.8	
P value comparing to baseline	0.005	0.04	
12 months	2.41±0.79	2.71±0.95	NS
Change from baseline	-0.99	- 0.66	
P value comparing to baseline	0.002	0.17	

could have employed special measures of support during and after treatment. Besides, since observed increments in contractility reported on several studies with cell therapy are small (in the range of 5 to 10%), the clinical impact of a small increase in heart function in a very ill population may be negligible. Due to these reasons, we can conclude that, in further studies, patients with LVEF<25% should be avoided. Secondly, we opted to analyze data by protocol instead of intention to treat, since patients who died had lower LVEF and maintaining their baseline data would have overestimated treatment effect. Thirdly, this was a non-blinded trial: it is possible that differences observed in quality of life and functional class could be due to placebo effect. Results from the 6-minute walk test would have been useful to assess more objectively functional capacity, but they were highly variable among patients, creat-

ing high values of standard deviation that precluded an appropriate analysis. Finally, high dispersion of data, from a wide range of parameters, such as LVEF and LV diameters, resulted in increased standard deviations in most outcome parameters, which in this small sample could have also precluded achievement of statistically significant differences between groups.

In conclusion, besides intragroup improvement in echocardiographic data, quality of life and NYHA class, when compared to control direct intramyocardial application of BMMC in NIDCM was not associated with sustained significant changes in left ventricular function. Functional capacity did not differ between groups. In future studies, less invasive forms of cell transplantation should be evaluated considering the high mortality observed, particularly for patients with severely impaired LVEF.

Authors' roles & responsibilities	
RTSA	Analysis and/or interpretation of data, statistical analysis, final approval of the manuscript, conception and design of the study, realization of operations and/or experiments, drafting of the manuscript and critical review of the content
JF	Analysis and/or interpretation of data, realization of operations and/or experiments
FHV	Analysis and/or interpretation of data, statistical analysis, final approval of the manuscript conception and design of the study, realization of operations and/or experiments, Drafting of the manuscript and critical review of the content
IC	Analysis and/or interpretation of data, realization of operations and/or experiments
NBN	Analysis and/or interpretation of data, statistical analysis, final approval of the manuscript, realization of operations and/or experiments
JRMSA	Analysis and/or interpretation of data, realization of operations and/or experiments
IAN	Analysis and/or interpretation of data, final approval of the manuscript conception and design of the study, realization of operations and/or experiments
RAKK	Analysis and/or interpretation of data, statistical analysis, final approval of the manuscript conception and design of the study, realization of operations and/or experiments, drafting of the manuscript and critical review of the content

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