Autologous Transplantation of Bone Marrow Adult Stem Cells for the Treatment of Idiopathic Dilated Cardiomyopathy

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Abstract

Background: Morbimortality in patients with dilated idiopathic cardiomyopathy is high, even under optimal medical treatment. Autologous infusion of bone marrow adult stem cells has shown promising preliminary results in these patients.

Objective: Determine the effectiveness of autologous transplantation of bone marrow adult stem cells on systolic and diastolic left ventricular function, and on the degree of mitral regurgitation in patients with dilated idiopathic cardiomyopathy in functional classes NYHA II and III.

Methods: We administered 4.54 x 10^8 ± 0.89 x 10^8 bone marrow adult stem cells into the coronary arteries of 24 patients with dilated idiopathic cardiomyopathy in functional classes NYHA II and III. Changes in functional class, systolic and diastolic left ventricular function and degree of mitral regurgitation were assessed after 3 months, 6 months and 1 year.

Results: During follow-up, six patients (25%) improved functional class and eight (33.3%) kept stable. Left ventricular ejection fraction improved 8.9%, 9.7% e 13.6%, after 3, 6 and 12 months (p = 0.024; 0.017 and 0.018), respectively. There were no significant changes neither in diastolic left ventricular function nor in mitral regurgitation degree. A combined cardiac resynchronization and implantable cardioversion defibrillation was implanted in two patients (8.3%). Four patients (16.6%) had sudden death and four patients died due to terminal cardiac failure. Average survival of these eight patients was 2.6 years.

Conclusion: Intracoronary infusion of bone marrow adult stem cells was associated with an improvement or stabilization of functional class and an improvement in left ventricular ejection fraction, suggesting the efficacy of this intervention. There were no significant changes neither in left ventricular diastolic function nor in the degree of mitral regurgitation. (Arq Bras Cardiol. 2014; 103(6):521-529)

Keywords: Cardiomyopathy, Dilated; Stem Cells; Transplantation, Autologous.

Introduction

Dilated Cardiomyopathy (DCM), the most common cardiomyopathy, is characterized by dilation of the left ventricle (LV) or both ventricles, eccentric hypertrophy, and systolic dysfunction[1]. Ventricular dilation, which is always present, can be mild, moderate, or severe, with left ventricular internal dimensions of up to 9.0 cm; decreased LV contractility may also occur to mild, moderate, and severe degrees, with or without pulmonary and/or systemic congestion symptoms. It is not uncommon for ventricular dilation to precede signs and symptoms of heart failure (HF). In approximately 50% of patients with DCM, the etiology cannot be identified; in these cases, patients are classified as having idiopathic DCM (IDCM)[2].

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Optimized medical therapy reduces the morbidity and mortality of patients with IDCM of New York Heart Association (NYHA)[3] functional class II or III and clinical stage C of the American College of Cardiology/American Heart Association (AHA/ACC)[4,5] classification according to disease progression. However, because this is a severe progressive disease with high mortality, drug treatment only slows progression to NYHA functional class IV in a significant number of patients, leading to early death or cardiac transplantation[6,7]. Therefore, there is an ongoing need for new and better methods of treating HF. Few trials in the literature have evaluated the efficacy of stem cell transplantation in treating patients with IDCM; however, the available results are promising[8].

This study aimed to determine the effects of autologous transplantation of adult bone marrow stem cells (BMSCs) on systolic and diastolic LV function and on the degree of mitral insufficiency (MI) in patients with IDCM in NYHA functional classes II and III.

Methods

This study was approved by the Ethics Committee on Human Research at the Hospital de Clinicas, Federal University

of Paraná (UFPR), registered under process BANPESQ 2005016327, and recorded in CEP/HC 1001.040/2005/03. All patients participating in the study signed the free and informed consent form.

Between June 2007 and June 2013, 24 patients (mean age 51.4 ± 11.5 years, 70.8% male) with IDCM were prospectively studied. The patients were stable but had HF NYHA functional classes II and III, despite at least 3 months of optimized medical treatment, and underwent autologous transplantation of adult BMSC via intracoronary infusion.

The study included patients aged between 30 and 75 years, whose transthoracic Doppler echocardiograms demonstrated left ventricular diastolic dimensions > 58 mm and LV ejection fraction (LVEF) ≤ 34% according to Simpson's modified method[9].

Exclusion criteria were as follows: presence of valvular disease, except mild to moderate functional MI or tricuspid insufficiency; coronary angiography showing ≥ 50% obstruction of one or more coronary arteries; seropositive status for Chagas disease or human immunodeficiency virus (HIV); current or previous use of cardiotoxic chemotherapy; abuse of alcohol or illegal drugs; history of sustained ventricular tachycardia; comorbidities impacting 2-year survival; and poor acoustic window for thoracic imaging.

Clinical evaluations were performed (anamnesis with special attention to determination of the functional class and physical examination, including anthropometric measurements) along with laboratory testing [hemogram, fasting blood glucose, creatinine, urea, potassium, uric acid, cholesterol, high-density lipoprotein cholesterol (HDL cholesterol), triglycerides, transaminases, creatinine phosphokinase (CPK), thyroid-stimulating hormone (TSH), and free T4], electrocardiogram, and Doppler echocardiography in the pre-transplant period and 3 months, 6 months, and 1 year after transplantation, with annual follow-up thereafter.

Doppler ecocardiography protocol

Complete and standardized Doppler ecocardiographs were performed on an outpatient basis in the Cardiology Methods Service at Hospital das Clínicas at UFPR by a single operator with level 3 training in accordance with the guidelines established by the ACC/AHA consensus[10]. The echocardiographies were performed using a Hewlett Packard Sonos® 5500 model or a Philips Envisor® (Bothell, Seattle, WA, United States) with a 2.5-MHz sectorial transducer, utilizing recommended plans in terms of M mode, two-dimensional and Doppler (pulsed, color and tissue), with simultaneous and continuous recording of the electrocardiogram. Among the variables studied and detailed below, we used the average of three measurements.

The left ventricular systolic function was evaluated by means of LVEF, calculated using the modified two-dimensional biplane Simpson's method (Figure 1). Values ≥ 55% were considered normal[9,11]. As an indirect parameter of left ventricular systolic function, in M mode, the distance in millimeters was measured between E point in the mitral valve ultrasound, corresponding to the initial opening of the mitral valve, and the anterior ventricular septum in its maximum posterior movement. A maximum value of 6 mm was considered normal[12].

Left ventricular diastolic function was assessed by measuring the velocities of the diastolic E waves [NV = 60 (50–90) cm/s] and A waves [NV = 50 (40–90) cm/s] of the mitral diastolic flow, the E/A wave ratio [NV = 1.2 [0.8–1.8)], and the deceleration time of the E wave (DT − NV = 217 (178–187) ms], measured from the interval between the E wave peak and the extrapolated intersection between the flow deceleration and the base line[13].

Using tissue Doppler, the the velocities of basal septal mitral ring movement waves were measured: systolic S (NV = 5.97 ± 1.14 cm/s), initial diastolic e' (NV = 7.91 ± 2.16 cm/s), and final diastolic a' (NV = 5.99 ± 1.73 cm/s). From this, the E/e' ratio was calculated (Figure 2)[14]. When atrial fibrillation rhythm was present, the E and e' wave measurements were performed over three consecutive beats, with cycles lasting between 10% and 20% of the mean cardiac rate.

The maximum volume of the left atrium was determined using Simpson's method, excluding the left atrial appendage and the mouth of the pulmonary veins and considering the mitral ring plane as the lower atrial border (Figure 3). This volume was divided by the body surface area, resulting in the left atrial volume index (LAVI)[9,15]. The normal value for LAVI is 22 ± 6 mL/m^2; however, because of its correlation with cardiovascular events, 32 mL/m^2 was used as the upper limit for normal[16-19].

Representing systolic and diastolic functions, the myocardial performance index, or Tei index, was defined as the ratio between the total isovolumetric times (contraction and relaxation) and left ventricular ejection time (NV = 0.34–0.44). This was calculated by subtracting the time between the beginning and the end of the systolic aortic flow from the time elapsed between the closing and the opening of the mitral valve, and dividing this result by LV ejection time (figure 4)[20].

MI was quantified by color Doppler using planimetry to obtain the outer border of the mitral regurgitant jet within the left atrium. The severity of MI was evaluated by the ratio between the area of the regurgitant jet and the area of the left atrium (ARJ/ALA): a relationship of <20% indicated discrete MI, 20%–40% indicated moderate MI, and >40% indicated significant MI (Figure 5)[21].

Bone marrow collection technique

The bone marrow was collected in the surgical center of the UFPR Hospital das Clínicas. In total, 30 mL of peripheral blood was collected to obtain autologous serum. The bone marrow was collected through multiple punctures (20) in both posterior iliac crests, always in distinct points. Approximately 5 mL was aspirated from each puncture in an attempt to reduce the contamination of the bone marrow with peripheral blood, totaling approximately 100 mL of bone marrow. The collected material was homogenized using Roswell Park Memorial Institute medium (RPMI medium) with the addition of penicillin 200 UI/mL and streptomycin 100 μg/mL as well as heparin sodium 350 units, in a proportion of one part bone marrow and one part medium. The tubes holding the bone marrow were sent for cell processing. The percentage

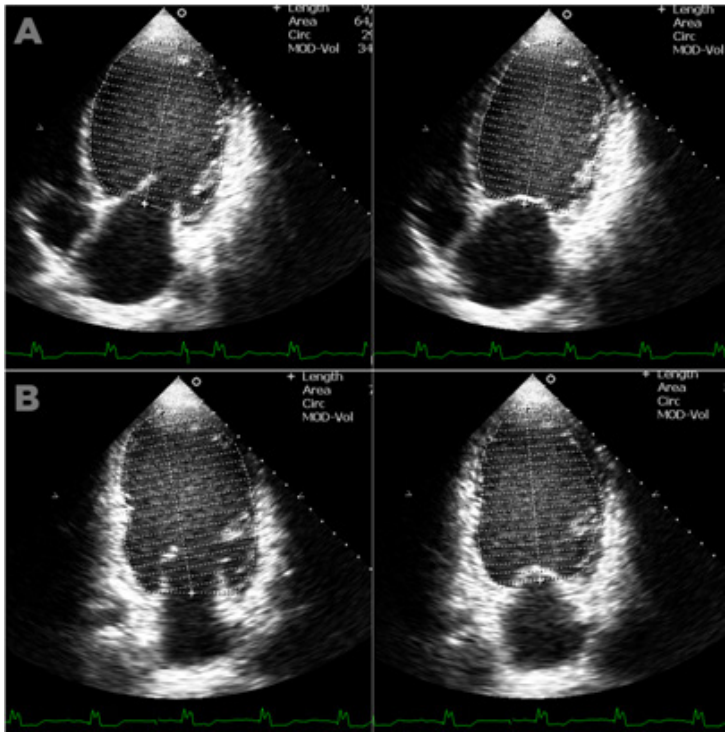

Figure 1 – *Modified Simpson's method to calculate the ejection fraction of the left ventricle (A) Four-chamber view; (B) two-chamber view; on the left, diastole; on the right, systole.*

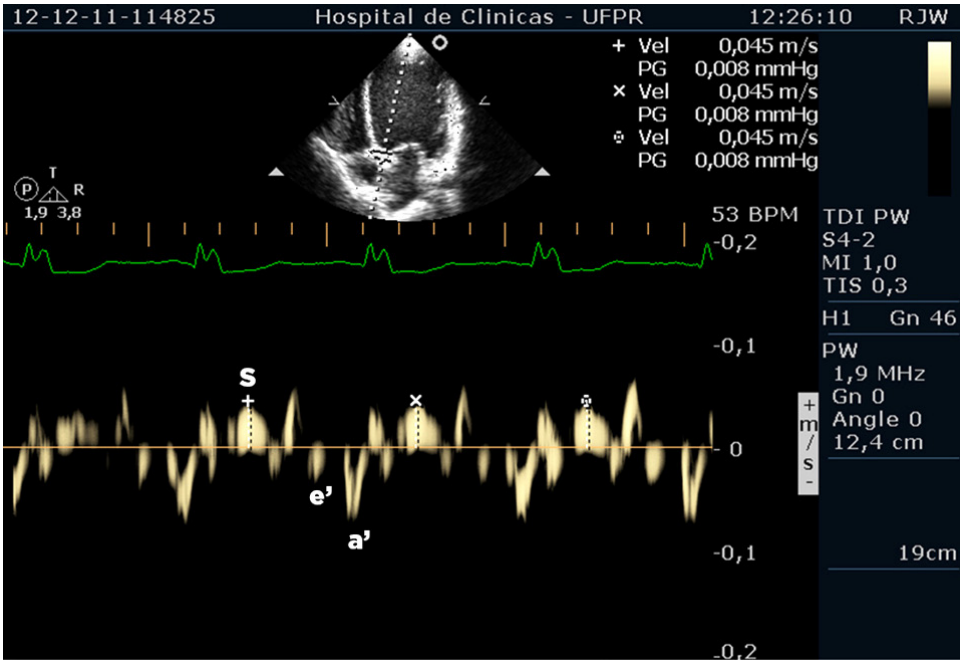

Figure 2 – *Tissue Doppler with the sample volume positioned in the mitral valve ring plane, in the septal wall S: systolic wave; e': early diastolic wave; a': atrial systolic wave.*

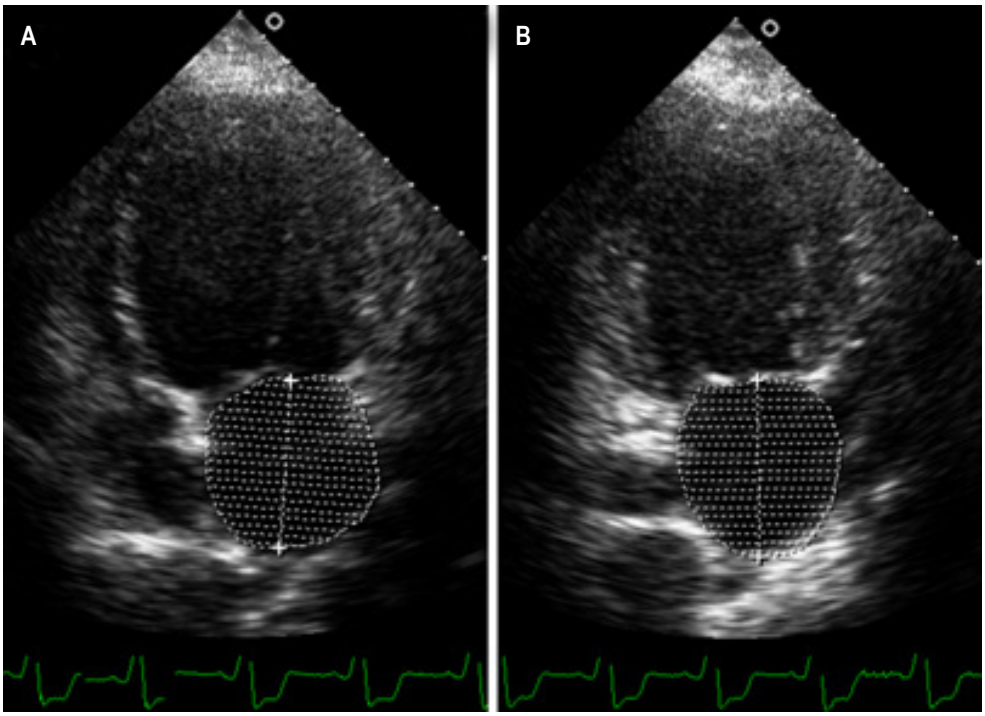

Figure 3 – *Modified Simpson's method to calculate to calculate the left atrial volume (A) Four-chamber view; (B) two-chamber view.*

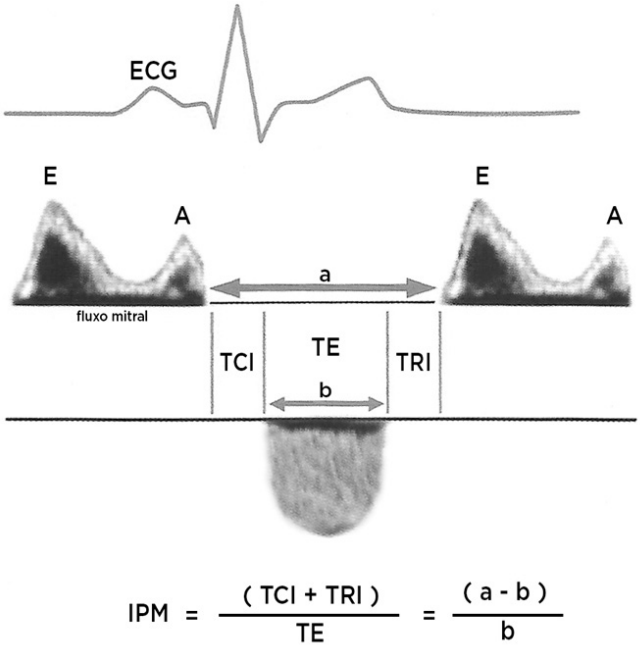

Figure 4 – *Method of calculating the myocardial performance index (IPM), or Tei index. ECG: electrocardiogram; TCI: isovolumetric contraction time; TRI: isovolumetric relaxation time; TE: ejection time.*

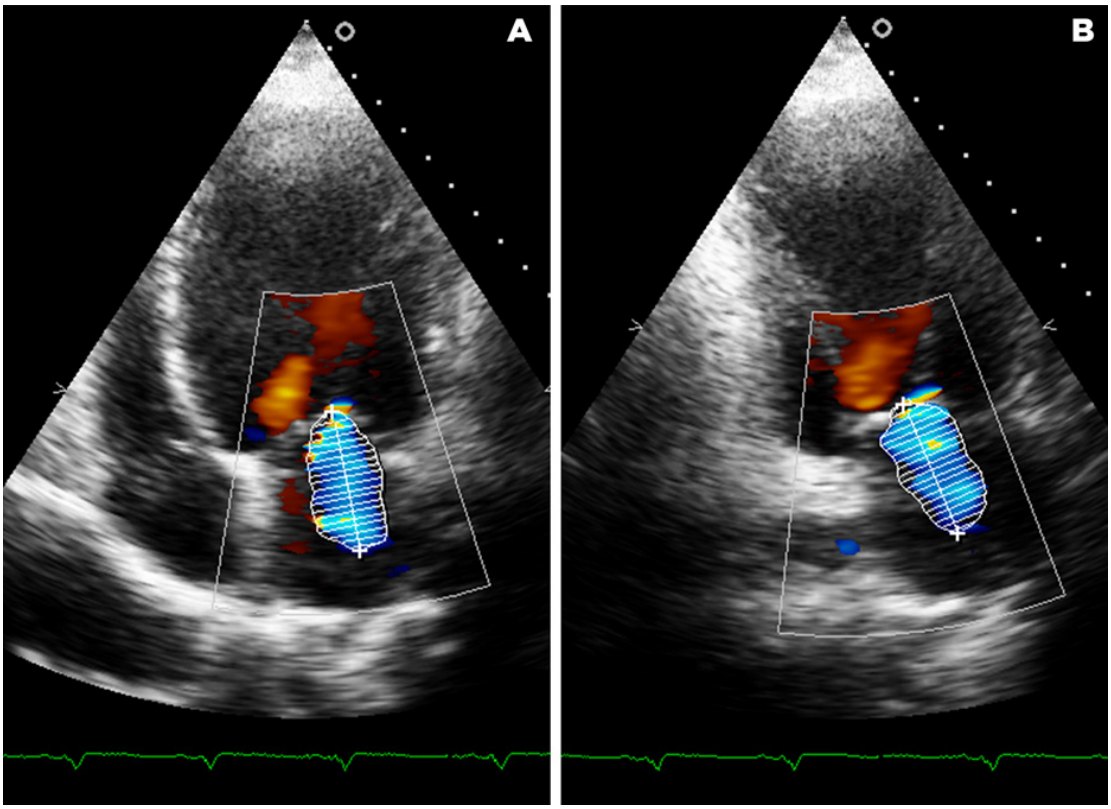

Figure 5 – *Area of the regurgitant jet (ARJ) in relation to the area of the left atrium (ALA) in a case of significant mitral insufficiency (ARJ/ALA > 40%).*

of viable cells was determined by homogenization of 0.5 mL of the cell suspension with 0.1 mL of a Trypan blue 0.4% solution, followed by a count of at least 100 cells in a Neubauer chamber. The adult BMSCs were used on the patients on the same day they were collected.

Intracoronary stem cell implant technique

Initially, coronarography using the Seldinger technique was performed to confirm the absence of coronary lesions and to describe the coronary arteries. It was followed by an infusion of autologous stem cells in the coronary arteries at a rate of 1 mL/min through an infusion catheter (AMICATH) positioned sequentially, using a 0.014'' guidewire, at the beginning of the left anterior descending (into which 10 mL of the solution was infused), the left circumflex (5 mL), and the right coronary artery (5 mL).

Statistical analysis

The results of quantitative variables were described as means ± standard deviations. Qualitative variables were described by frequencies and percentages. The normality of the variables was analyzed using the Kolmogorov–Smirnov test. For each variable evaluated, each post-transplant evaluation was compared with the pre-transplant evaluation using the Student's *t* test for paired samples. Considering the first four assessment periods (pre-transplant and 3 months, 6 month, and 1 year after transplantation) for each variable, a patient's profile analysis was performed, assessing, assessing the homogeneity of patient progress over the period of 1 year. The patient survival time after transplantation was described by means of a Kaplan–Meier curve. p values < 0.05 indicated statistical significance. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 20.0.

Results

In the initial clinical evaluation, 10 patients (41.7%) were in NYHA functional class II, while 14 (58.3%) were in functional class III. The mean LVEF was 27.08 ± 5.12% (17%–34%). The basal electrocardiogram of five patients (20.8%) did not present conduction disturbances; four patients (16.6%) had left bundle branch conduction disorder; 15 patients (62.5%) presented with left branch block.

On average, $4.54 \times 10^8 \pm 0.89 \times 10^8$ (2.11×10^8–5.06×10^8) adult BMSCs with 93.04% viability were injected into the coronary arteries. There were no complications related to bone marrow aspiration or the intracoronary infusion of adult BMSCs. In addition, these procedures revealed no clinical or electrocardiographic changes suggestive of myocardial ischemia.

In the long term after adult BMSC infusion, six patients (25%) showed an improvement from NYHA functional class III to II, while eight patients (33%) remained stable in the same functional class without complications. Five patients (20.8%) showed clinical complications with worsening in functional class: one due to renal failure after cholecystectomy, one due to bronchial pneumonia and pleural effusion, and one due to acute atrial fibrillation rhythm reverted using electrical cardioversion. The other two patients (8.3%) received cardiac resynchronization therapy: one for the worsening of HF and the other for nonsustained and sustained ventricular tachycardia. With clinical management, four of these patients returned to their initial functional class, while one patient improved by one functional class.

During the follow-up period, eight (33.3%) deaths occurred; of these, four (16.6%) patients had sudden cardiac death and four (16.6%) terminal HF. Figure 6 shows the graph of the Kaplan–Meier survival curve for the sample studied.

With regard to the echocardiographic parameters observed, compared with pre-transplant levels, a continuous and significant improvement in LVEF was observed during clinical follow-up, with an average increase of 8.9%, 9.7%, and 13.6% after 3 months, 6 months, and 1 year, respectively ($p < 0.05$ for all). This occurred at the expense of a significant decrease in the LV end systolic volume without a significant change in the end diastolic volume. The distance between E point and the ventricular septum in M mode, significantly reduced in the first 3 months after transplantation and remained stable during later follow-up. DT, in turn, significantly increased during the 3-month period, returning to the base levels and remaining stable. The remaining parameters (LAVI, ARJ/ALA, E/A ratio, E/e' ratio, and Tei index) showed no statistically significant variations in comparisons between pre-transplant values and the various post-transplant stages (Table 1).

Discussion

The benefit of stem cells in the treatment of cardiovascular diseases is related to possible neovascularization and myocardial tissue formation because of these cells' ability to acquire the characteristics of other cell lines such as myocytes, vascular smooth muscle cells, and endothelial cells[22,23]. In addition, adult BMSCs injected into the coronary arteries can indirectly contribute to cardiac regeneration by releasing several peptides, which have a paracrine effect on the myocardium and the resident cardiac progenitor cells[24,25].

Clinical and experimental studies[26-33] suggest benefits from stem cell therapy in ischemic and Chagas-related DCM, mainly because of a decrease in fibrosis and an increase in vasculature but without concrete evidence of transdifferentiation of stem cells into cardiomyocites. However, these studies did show a significant improvement in the functional class and left ventricular systolic function, as assessed by LVEF. Seth et al.[8] performed intracoronary stem cell transplantation in patients with DCM, obtaining an improvement in the functional class and a significant 5.4% increase in LVEF in the treated group over a 6-month follow-up period. Vrtovec et al[28] performed intracoronary CD34 stem cell transplantation in 28 patients with IDCM guided by contractile alterations accented by scintillography, with a control group. After 1 year, patient LVEF increased from 25.5 ± 7.5% to 30.1 ± 6.7% ($p = 0.03$), the distance in the 6-min walk test increased, and the level of the natriuretic peptide NT-proBNP decreased, along with potential increased survival; this result persisted during the 5-year follow-up period[29]. Vilas-Boas et al.[33] conducted stem cell transplantation in 28 patients with Chagas-related HF and demonstrated in increase in LVEF from 20.1 ± 6.8% (base) to 28.3 ± 7.9% ($p < 0.03$) 6 months after the procedure. In our sample, an improvement or stabilization of the functional class was observed in a significant number of patients, in addition to an important evolutionary improvement in LVEF compared with pre-transplant base values, with an average increase of 8.9%, 9.7%, and 13.6% after 3 months, 6 months, and 1 year, respectively; these values persisted until 3 years after the procedure. Consistent with the findings in the literature[8], this increase was due to a reduction in ESV and stability of EDV and SV.

The E-SV parameter demonstrated a discreet decrease between the pre-procedure period and 3 months post-procedure but remained stable during follow-up and comparisons between pre-procedure and 6 months post-procedure and pre-procedure and 1 year post-procedure. It is known that this parameter increases with decreased ejection fraction, possibly as a result of the remodeling of the ventricular cavity with increasing diameters, primarily the anteroposterior and ventricular volumes. However, in this study, we did not observe any increase, probably because of the presence of left bundle branch block, an intraventricular conduction disturbance, and intraventricular dyssynchrony, which was present in 19 (79.2%) of the 24 patients[9].

The E/A ratio of the diastolic mitral flow waves, the E wave DT in early mitral diastolic flow, the E/e' ratio, and the LAVI by body surface area are all echocardiographic indices that assess LV diastolic function. No significant sustained changes occurred in these variables, reflecting nonsignificant progress in the diastolic function of the study population.

In addition, there was no significant variation in the Tei index. In patients with IDCM, this index, which reflects left ventricular systolic and diastolic performance, has independent prognostic value if it worsens, which, which did not occur in this study.

The semi-quantitative assessment of MI severity did not show statistical significance in the comparisons between the pre-transplant evaluations and subsequent evaluations until 3 years post-procedure. Mild to moderate MI in the pre-transplant period did not progress to moderate or severe levels at any time, and it is interpreted as non-worsening in left ventricular remodeling, which is the cause of mitral reflux in IDCM because of mitral ring dilation.

Joint analysis of these indices allows us to infer that on average, our patients exhibited grade III diastolic dysfunction, named reversible restrictive pattern. These results, along with the Tei index, corroborated the severity of diastolic and systolic

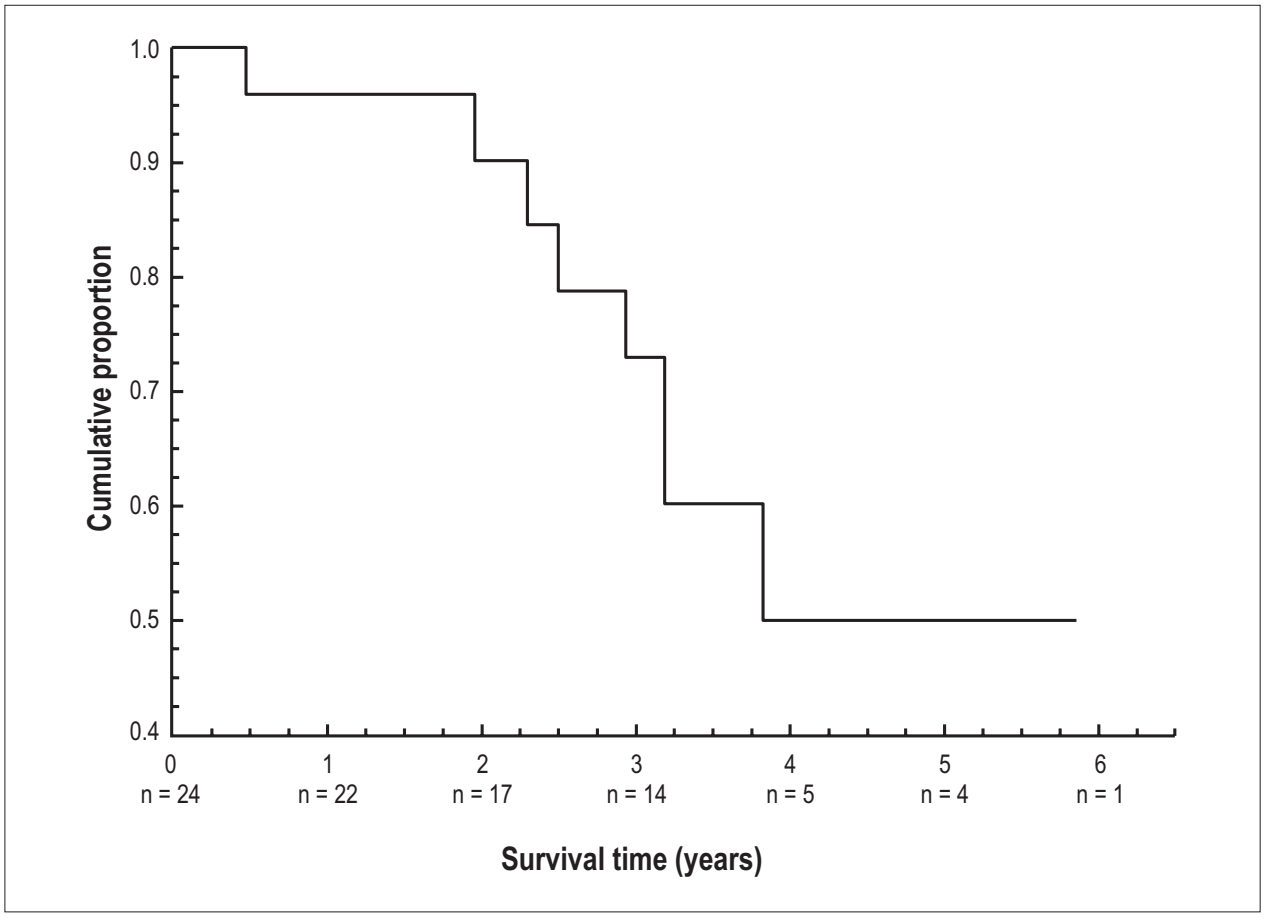

Figure 6 – *Kaplan–Meier survival curve for the sample studied*

Table 1 – Echocardiographic parameters at baseline and follow-up

Variable	Pre-transplant (n = 23)	3 months (n = 23)	6 months (n = 23)	1 year (n = 22)	2 years (n = 13)	3 years (n = 9)
LVEF (%)	26.91 ± 5.16	29.30 ± 6.04*	29.57 ± 6.54*	30.41 ± 7.85*	33.54 ± 8.14*	36.11 ± 10.06*
ESV (mL)	212.65 ± 68.34	200.48 ± 77.61*	191.91 ± 78.46*	201.36 ± 82.37*	179.62 ± 96.08*	160.44 ± 93.03*
EDF (mL)	288.48 ± 80.60	278.39 ± 88.26	267.61 ± 89.04*	283.23 ± 91.70	260.23 ± 115.55	237.67 ± 102.4*
SV (mL)	75.87 ± 19.95	77.87 ± 17.67	75.87 ± 18.54	82.00 ± 19.46	80.31 ± 22.91	77.22 ± 14.74
E-SV (mm)	25.48 ± 5.36	24.57 ± 4.90*	24.70 ± 6.14	25.05 ± 5.39	26.69 ± 5.59	22.78 ± 7.63
LAVI (mL)	59.17 ± 17.42	54.26 ± 20.74	54.22 ± 22.51	57.23 ± 20.42	60.69 ± 27.52	50.44 ± 22.37
E/A	1.44 ± 0.66	1.37 ± 0.99	1.43 ± 1.04	1.71 ± 1.13†	1.34 ± 0.94‡	1.06 ± 0.75§
DT (ms)	203.26 ± 48.02	243.81 ± 101.98*	205.91 ± 79.32	213.45 ± 83.025	214.69 ± 55.14	223.11 ± 87.19
E/e'	12.83 ± 4.76	12.13 ± 3.79	12.74 ± 4.49	13.59 ± 4.81	11.69 ± 3.68	12.11 ± 3.44
Tei index	0.93 ± 0.25	0.86 ± 0.27	0.87 ± 0.36	0.84 ± 0.33	0.98 ± 0.31	0.92 ± 0.32
ARJ/ALA	26.34 ± 11.85	26.00 ± 9.70	26.33 ± 10.94	30.06 ± 12.75	22.72 ± 7.81	26.24 ± 11.56

*LVEF: left ventricular ejection fraction; ESV: end systolic volume; EDV: end diastolic volume; SV: systolic volume; E-SV: distance between E point and the ventricular septum in M mode; LAVI: left atrial volume index; E/A: relationship between E and A wave speeds in diastolic mitral flow; DT: E wave deceleration time; E/e': relationship between E wave in diastolic mitral flow and e' wave movement in the septal basal area of the mitral ring; ARJ/ALA: ratio between the area of the mitral regurgitant jet and the area of the left atrium. *p < 0.05 in comparison with pre-transplant values; †n = 21, as one patient developed unreverted atrial fibrillation; ‡n = 12; §n = 8.*

dysfunction in the population studied as well as their high mortality demonstrated in the Kaplan–Meier survival curve, with an average survival of 2.6 years for the eight patients who died.

IDCM is the main substrate for sudden cardiac death. The absolute risk of sudden death increases with deterioration of cardiac function; however, the relationship between sudden and non-sudden deaths is inversely related to the extent of the functional damage, i.e., mortality is lower but the likelihood of sudden death is greater among patients with cardiomyopathy in better functional classes (I and II). The mortality rate observed in our population is similar to that obtained by Vrtovec et al.[29] These authors found an overall mortality rate of 25% (48% because of HF and 52% because of sudden cardiac death) 5 years after intracoronary stem cell transplantation, although this was lower in the group that received stem cells (14% vs. 35%; p = 0.01). The same occurred with the deaths from HF (5% vs. 18%; p = 0.03) but not with sudden cardiac death (9% vs. 16%; p = 0.39)[29].

The limitations of this study are as follows: the absence of a control group only in optimized clinical treatment, the lack of prior endomyocardiac biopsy to rule out other possible causes of DCM, and the fact that analysis of the echocardiographic parameters was performed by a single qualified operator.

Conclusion

This study demonstrated that patients with DCM who underwent intracoronary implant of autologous stem cells remain stable or improve their NYHA functional class and demonstrate significant improvement in left ventricular systolic function because of increased LVEF. Consequently, it may be a stimulus for additional research on the application of stem cells in IDCM.

Author contributions

Conception and design of the research and Statistical analysis: Westphal RJ; Acquisition of data: Westphal RJ, Bueno RRL, Galvão PBA, Zanis Neto J, Souza JM, Senegaglia AC; Analysis and interpretation of the data: Westphal RJ, Bueno RRL, Guérios EE, Senegaglia AC; Obtaining financing: Westphal RJ, Pasquini R, Cunha CLP; Writing of the manuscript: Westphal RJ, Guérios EE; Critical revision of the manuscript for intellectual content: Westphal RJ, Bueno RRL, Zanis Neto J, Souza JM, Guérios EE, Brofman PR, Pasquini R, Cunha CLP.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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