Bone Marrow Cell Transplantation to the Myocardium of a Patient with Heart Failure Due to Chagas' Disease

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We report the first case of bone marrow cell transplantation to the myocardium of a patient with heart failure due to chagas' disease. The patient is a 52-year-old man with chronic heart failure, NYHA functional class III, despite the optimized clinical therapy. The procedure consisted of aspiration of 50 mL of bone marrow through puncture of the iliac crest, followed by filtration, separation of the mononuclear cells, resuspension, and intracoronary injection. The left ventricular ejection fraction at rest, measured using radionuclide ventriculography with labeled red blood cells prior to transplantation, was 24%, and, after 30 days, it increased to 32% with no change in the medicamentous schedule. The following measurements were assessed before and 30 days after transplantation: left ventricular end diastolic diameter (82 mm and 76 mm, respectively); Minnesota living with heart failure questionaire score (55 and 06, respectively); and distance walked in the 6-minute walking test (513 m and 683 m, respectively). Our findings show that intracoronary injection of bone marrow cells may be performed, suggesting that this is a potentially safe and effective procedure in patients with due to Chagas' disease heart failure.

Heart failure is epidemic at the beginning of this century. In Latin America, an endemic region for Chagas' disease with approximately 11 million people suffering from the disease, the situation is even worse[1]. In chagasic patients, no specific etiologic treatment after heart failure syndrome is in place has yet been proven efficient. Therefore, the treatment of these patients does not differ from that of patients with heart failure due to other etiologies[2].

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Given the limited efficacy of the current pharmacological therapeutic alternatives for the treatment of heart failure[2], other forms of treatment have been developed[3]. The demonstration of the capacity of adult bone marrow cells to differentiate *in vitro* into various cell types was the initial stimulus for their experimental use in the treatment of heart failure[4].

Preliminary studies on the use of bone marrow cells for the treatment of chronic chagasic cardiomyopathy have been carried out at the Gonçalo Moniz Research Center (FIOCRUZ-BA) using a mice model infected by *Trypanosoma cruzi*, which showed a significant reduction in inflammation and a regression in fibrosis after 2 months of treatment with adult bone marrow cells as compared with that in control animals[5].

This and other findings[6-8] served as a basis for designing a protocol for the use of bone marrow cell therapy in patients with chagasic cardiomyopathy.

Case report

The patient is a 52-year-old man diagnosed 3 years previously with heart failure of chagasic etiology. The patient was stable, despite severe functional limitation, using digoxin (0.25 mg/d), enalapril (10 mg BID), furosemide (40 mg/d), spironolactone (25 mg/d) and carvedilol (6.25 mg BID). His blood pressure was 120/80 mmHg, his heart rate was 64 bpm, his respiratory rate was 18 ipm, and his weight was 61 kg. The patient had bilateral jugular stasis, his lungs were clear, the intensity of the cardiac sounds was normal, and a II/VI-degree mitral holosystolic murmur could be heard radiating to the axilla, with no S3. The liver border was under the right costal margin, and no peripheral edema existed. The 12-lead electrocardiogram revealed sinus rhythm, complete right bundle-branch block and anterosuperior divisional block. The chest radiography showed cardiomegaly. The routine biochemical and hematological tests were normal as follows: ALT, 33.6 UI/L; AST, 37.5 UI/L; sodium, 136 mEq/L; potassium, 4.4 mEq/L; glucose, 83 mg/dL; hemoglobin, 15.6 g/dL.

The procedure was performed with the patient sedated, and comprised 5 punctures of the crest of each iliac bone, from where approximately 50 mL of bone marrow where withdrawn. The material was passed through a bone marrow filtering system (Washington University® - USA), and, then, the mononuclear cells were separated through a Ficol gradient. After resuspension in 20 mL of albumin saline solution (5%), approximately 2.4 x 10^8 cells were injected in the right and left coronary system, through an angioplasty catheter as follows: 10 mL of the suspension were slowly injected in the anterior descending coronary artery for 10 minutes; 5 mL in the circumflex artery; and 5 mL in the right coronary artery. Coronary artery disease was excluded through coronary angiography. The patient experienced neither arrhythmias nor electrocardiographic alterations during the procedure. The vital data remained stable during the entire period of hospitalization (tab. I).

Alterations were not observed in the markers of myocardial necrosis nor in the biochemical and hematological parameters (tab. II). After 4 days, the patient was discharged using the same medications used prior to the procedure in addition to ascorbic acid (1g/d). In the initial 30-day follow-up, weekly clinical reassessments were performed, as were laboratory assessments at the end of the period.

A reduction in the ventricular diameters was observed on echocardiography and there was a significant increase in the left ventricular ejection fraction both on echocardiography and radionuclide ventriculography with labeled red blood cells. Functional capacity, assessed both with the NYHA functional classification and 6-minute walking test, showed a significant improvement, as did the quality of life score (tab. III).

Table I - Evolution of vital data after bone marrow cell transplantation

	Basaline	30 min	1h	6h	12h	24h
SBP (mmHg)	120	120	110	100	103	96
DBP (mmHg)	80	70	60	48	71	45
HR (bpm)	68	60	52	65	72	85
RR (bpm)	18	17	18	18	17	15

SBP - systolic blood pressure; DBP - diastolic blood pressure; HR- heart rate; RR - respiration rate.

Table II - Laboratory evolution of hematological and biochemical parameters and of myocardial necrosis markers after bone marrow cell transplantation

	Basaline	24h	72h	1 month
Hemoglobin (g/dL)	15.6	14.5	15.3	14
Hematocrit (%)	45.3	42.2	44.00	42.6
Total leukocytes (u/mm^3)	7.020	5.200	6.710	8.070
Platelets (u/mm^3)	334.00	272.00	260.000	271.00
Troponin I (ng/mL)	<0.01	0.012	-	-
CK-MB (UI/L)	16.00	3.58	-	-
Urea (mg/dL)	23.6	20	22	22
Creatinine (mg/dL)	0.87	0.90	1.10	1
AST (UI/L)	42.2	33.6	69.1	18.1
ALT (UI/L)	35.6	37.5	84.2	20.9
Sodium (mEq/L)	136	134	134	139
Potassium (mEq/L)	4.4	4.4	4.2	4.2

Discussion

Chagasic cardiomyopathy is one of the major causes of heart failure in Latin America, and its clinical presentation is similar to other forms of dilated cardiomyopathy. The chronic phase of the disease is characterized by a multifocal inflammatory infiltration of mononuclear cells. In this phase, no association exists between the presence of parasites (*Trypanosoma cruzi*) and the intensity of inflammation or fibrosis[9].

In recent decades, several therapeutic options have been developed or improved to delay the progression of ventricular dysfunction in patients with heart failure. However, plain reversion of the process has not yet been obtained, and the prognosis of these patients remains very limited.

Cell therapy has emerged as a promising option for the treatment of advanced cases of heart failure. Orlic et al[6] , in a study with infarcted mice, reported myocardial repair through the formation of new cardiac fibers and neoangiogenesis after bone marrow cell transplantation. Strauer et al[7] were the first to demonstrate the feasibility and safety of intracoronary infusion of bone marrow stem cells for the treatment of patients with ischemic cardiomyopathy. Perin et al[8] showed, for the first time, the implatation of bone marrow mononuclear cells to the myocardium of patients with ischemic heart failure with no possibility of percutaneous or surgical revascularization. Using a technique of electromechanical endomyocardial mapping (NOGA), the cells were injected via the endocardial route in the periphery of the ischemic region, resulting in improvement in clinical and laboratory parameters.

Our's the first report of bone marrow cell transplantation to the heart of a patient with chagasic heart failure. The Chagas' disease model is particularly attractive for the use of stem cell therapy. Theoretically, howing of stem cells in the myocardium requires that some cytokine or chemotactic factor be produced, attracting the cells[3]. In chronic Chagas' heart disease, an elevated production of cytokines occurs due to persistent multifocal inflammation; therefore, these factors may account for cell attraction, fixation, and differentiation[9,10].

In contrast with previous studies, we injected approximately 10 to 20 times more cells (2.4 X 10^8) than what has

Table III - Evolution of the ventricular function, functional capacity, and quality of life

	Baseline	1 month	Variation %
LVEDD – echocardiogram (mm)	82	76	-7.3
LVEF – echocardiogram (%)	28	38	+35.0
LVEF – ventriculography (%)	24	32	+33.0
NYHA functional class	III	II	-
Distance walked in the 6-minute walking test(m)	513	683	+33
Minnesota quality of life score	55	06	-89

LVEDD- left ventricular end-diastolic diameter; LVEF- left ventricular ejection fraction.

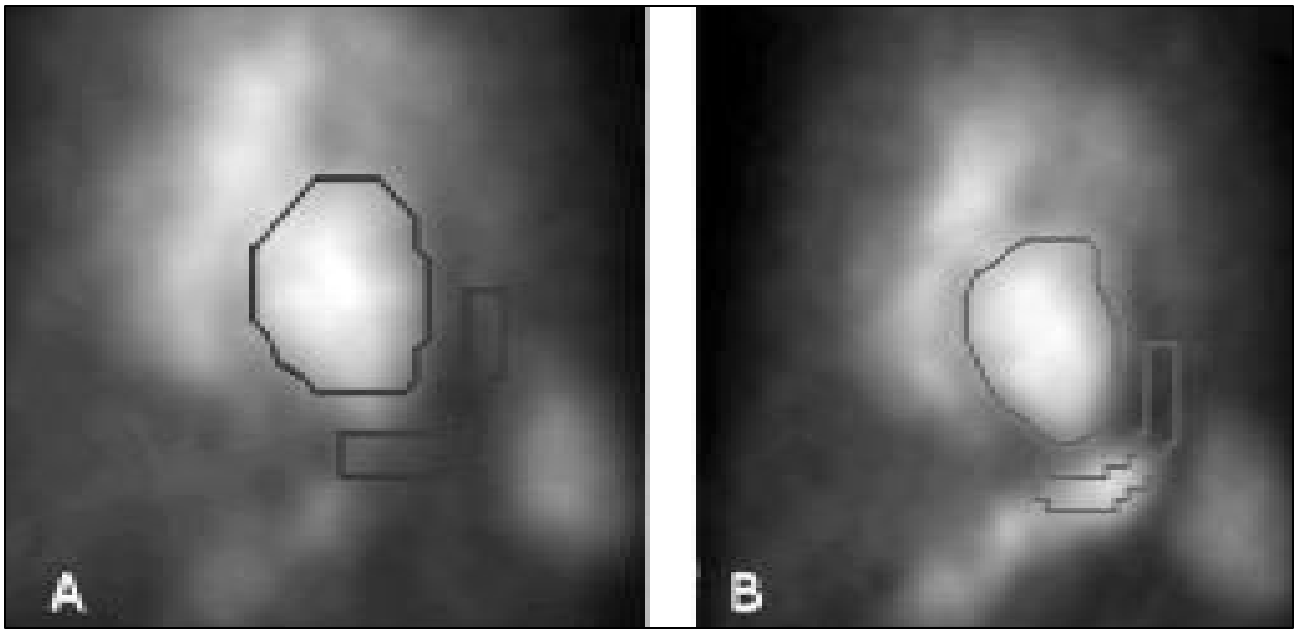

Fig. 1- Radionuclide ventriculography with labeled red blood cells pre (A) and post (B) bone marrow cells transplantation.

been reported in patients with ischemic cardiomyopathy[7,8]. The lack of adverse effects related to the infusion of a greater cell concentration may be partially attributed to the following 2 factors: 1) use of a slow-infusion regimen under low pressure; 2) absence of coronary artery disease in epicardial vessels or in the microcirculation.

The cell type used is worth noting. We used the technique of bone marrow aspiration and separation of mononuclear cells, among which were those recognized as stem cells. Another way of obtaining pluripotent cells is through stimulation of the bone marrow with granulocyte colony-stimulating factor (G-CSF), and removal, through serial aphereses, of populations of stem cells present in the peripheral blood. The theoretical disadvantage of removing cells from the peripheral blood lies in the fact that it has not yet been determined which cell lineage is responsible for the differentiation into cardiomyocytes; ie, another mononuclear cell lineage may differentiate into cardiomyocytes, or even into new vessels, nerves, etc. Another theoretical disadvantage of the use of peripheral blood cells is that the mobilized cells may have preferentially followed a hematopoietic lineage, while the cells that could differentiate into the cardiomyocyte lineage remained in the bone marrow.

The lack of adverse events related to the procedure indicates its safety. Improvement in ventricular function was consistently identified through 2 methods, one of which is the gold standard for assessing ejection fraction. In the absence of changes in the therapeutic regimen, and with the patient stable for more than 2 months, we found no explanation for the improvement in ventricular function other than cell therapy. The other parameters of improvement (functional capacity, quality of life, functional class) should be carefully analyzed, especially because they are subjective variables influenced by the patient's motivation.

The case reported showed that intracoronary injection of transplanted bone marrow cells can be performed in patients with chagasic heart failure, suggesting that the procedure is potentially safe and effective. A larger case series is required to confirm whether the results observed can be reproduced and justify the beginning of phase II clinical trials.

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