Original Article

Early Results of Bone Marrow Cell Transplantation to the Myocardium of Patients with Heart Failure due to Chagas Disease

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OBJECTIVE

To evaluate early effects of bone marrow cell transplantation to the myocardium of patients with heart failure (CHF) due to Chagas disease.

METHODS

We studied 28 patients (mean age 52.2 ± 9.9), of whom 24 were male. Despite optimized treatment, 25 patients were in NYHA class III and three patients, in NYHA class IV. The procedure consisted of aspiration of 50 mL of bone marrow, separation of the mononuclear fraction, and intracoronary injection. Effects on left ventricle ejection fraction (LVEF), distance walked in the six-minute walking test, quality-of-life, NYHA class, arrhythmogenic and biochemical parameters, were all evaluated.

RESULTS

There were no complications directly related to the procedure. Baseline left ventricular ejection fraction was 20.1 ± 6.8%, and 60 days after transplantation it increased to 23.0 ± 9.0%, p = 0.02. Significant improvements were observed in the NYHA class (3.1 ± 0.3 to 1.8 ± 0.5; p < 0.0001); quality-of-life (50.9 ± 11.7 to 21.8 ± 13.4; p < 0.0001); and distance walked in six minutes (355 ± 136 m to 443 ± 110 m; p = 0,003). The number of ventricular premature beats in 24 hours tended to increase (5,322 ± 4,977 to 7,441 ± 7,955; p = 0,062), but without increase in ventricular tachycardia episodes (61 ± 127 to 54 ± 127; p = 0.27).

CONCLUSION

Our data demonstrate for the first time that intracoronary injection of bone marrow mononuclear cells is feasible and suggest that it may be potentially safe and effective in patients with CHF due to Chagas disease.

KEY WORDS

Stem cell, heart failure, Chagas disease, cell therapy, dilated cardiomyopathy.

The ability of bone marrow stem cells to differentiate into cardiomyocytes has been demonstrated especially in experimental models of myocardial infarction[1-3]. Although some degree of controversy regarding stem cell plasticity exists, such observations have led investigators to examine, in humans, their role in the management of myocardial infarction and heart failure of ischemic etiology, and the first results are promising[4-6].

In Brazil, Chagas disease is one of the main contributors to the nosological description of heart failure. Serological estimates show that eight or nine million Brazilians are infected by *Trypanosoma cruzi*, and that 30 to 40% of them may have some degree of cardiac involvement[7]. Patients with heart failure secondary to chronic Chagas heart disease progress to severe systolic dysfunction due to the chronic myocarditis and consequent fibrosis[8].

The etiopathogenic and pathophysiological mechanisms involved in Chagas disease make stem cell therapy attractive. Both the presence of persistent myocarditis and increased production of cytokines by the myocardium in patients with CHF due to Chagas disease suggest the existence of an attractive environment for stem cell homing and adhesion[9]. Therefore, unlike the management of other heart diseases in which little inflammation is found, in Chagas heart disease it may be assumed that circulating stem cells migrate to the myocardium, recruited by the inflammatory signals spread throughout the organ.

Experimental studies using stem cells derived from bone marrow to treat chronic Chagas cardiomyopathy demonstrate that, two month after treatment with adult bone marrow cells, chronic chagasic mice showed a significant reduction in inflammation and fibrosis, compared with control animals[10].

The first case of bone marrow stem cell transplantation to the myocardium of a patient with heart failure secondary to Chagas disease was reported by our group, leading to improvement in ventricular function, quality of life, and functional capacity[11].

This study was designed to assess the safety and feasibility of autologous bone marrow mononuclear cell (BM-MNC) transplantation in patients with heart failure due to Chagas disease, as well as identify early evidence supporting the effectiveness of the procedure.

METHODS

The study was an open-label, uncontrolled, single-center, phase-1 clinical trial carried out at the Santa Izabel Hospital of the Santa Casa de Misericórdia da Bahia, Brazil, involving selected patients with CHF secondary to Chagas disease.

Patients who met the following inclusion criteria were consecutively included in the study: subjects of both genders, ranging from 20 to 70 years, with CHF due to Chagas disease, left ventricle (LV) ejection fraction less than 40% on echocardiogram, functional class III and IV (NYHA), receiving optimal chronic treatment to CHF (digoxin, diuretics, angiotensin-converting enzyme inhibitors, with or without beta-blockers or angiotensin-II receptor antagonists), and who remained stable in this condition in the month prior to the experiment.

We excluded patients with associated systemic conditions, such as infections or neoplasias, autoimmune diseases, and neurodegenerative diseases; acute or acutely decompensated heart failure; previous hematologic diseases; coagulopathies; liver failure; moderate renal failure (creatinine above 2 mg/dL); previous history of chronic obstructive pulmonary disease (COPD); implantation of biventricular resynchronization pacemaker in the previous 90 days; women of childbearing potential, and patients with coronary artery disease detected previously or after coronary angiography.

This protocol was approved by CONEP (Brazilian National Committee for Ethics in Research) under No. 4108; Case No. 25000.054219/2002/20). Patients were fully informed about study procedures and, after reading and signing the informed consent, they were included in the study.

All patients underwent a baseline clinical evaluation, and the following clinical data and variables were recorded: a) functional class (NYHA), b) quality-of-life score of the "Minnesota Living With Heart Failure Questionnaire"; c) hematological and biochemical assessment; d) twelve-lead electrocardiogram; e) transthoracic echocardiogram; f) radionuclide ventriculography; g) six-minute walk test; g) 24-hour Holter. After the procedure, patients were referred to the Intensive Care Unit, where they were monitored for at least 24 hours. If everything went uneventfully, they were transferred to the regular ward where remained for a minimum of five days for in-hospital follow-up. All clinical evaluation, laboratory tests and complementary exams were repeated after 30 and 60 days.

To check for any possible myocardial damage caused by bone marrow cell injection, serial measurements (every six hours during the first 24 hours) of markers of myocardial damage (CK-MB and troponin I), as well as electrocardiograms, were performed. To investigate the development of cardiac arrhythmias as a complication of cell injection, patients underwent ambulatory electrocardiography (Holter) 24 hours before the procedure and periodically thereafter (after 24 hours, 30 days and 60 days).

Stem cell transplantation - In the morning before the procedure, patients were referred to the cath lab after overnight fasting. Fifty ml of bone marrow content were aspirated from every patient through five punctures in the right posterior iliac crest and five in the left posterior iliac crest, 5 ml per puncture, under local anesthesia with 2% lidocaine and intravenous sedation using midazolam. The harvested aspirate was filtered to remove bone marrow debris, such as fat and bone fragments (stainless steel mesh, Washington University), and centrifuged

by Ficoll Hystopaque gradient (Amersham Pharmacia, a product licensed for clinical use in humans). The isolated mononuclear cell fraction was then diluted in sterile saline solution and centrifuged again. One sample was used for cell count and viability test. At the end of the process, samples were diluted in 20 ml of saline solution. Immediately before intracoronary injection, patients underwent left heart catheterization by the femoral approach followed by coronary angiography. Patients with evidence of hemodynamically significant coronary disease (stenosis $\geq$ 50%) were excluded from the study. The cell solution was slowly injected, during 10 minutes, into the right and left coronary system. Ten ml of the solution was injected in the anterior descending artery, 5 ml in the right coronary artery, and 5 ml in the left circumflex artery.

Twenty-five days later, patients received daily subcutaneous injections of human G-CSF (Granulokine®) for five days, at a dose of 5 mcg/kg/day, to induce mobilization of bone marrow stem cells to peripheral blood, so that a greater number of stem cells would be available for injury repair. In studies using an experimental model of chagasic myocarditis, combined cell therapy and G-CSF treatment led to a significantly greater decrease in the number of inflammatory cells than cell therapy alone (unpublished data).

The six-minute walk test was performed according to the protocol used by Bitner *et al*. in the SOLVD study[12]. After a fifteen-minute rest, a new test was carried out, and the average of the distances walked in the two tests was used as the result. Quality of life was assessed by the "Living with Heart Failure Questionnaire" developed by the University of Minnesota[13] and validated by Carrara *et al.* in Brazil[14]. Echocardiographic studies were conducted by the same observer, blind to previous examinations, on a Hewlett-Packard Sonos 5500 System (Seattle, WA, EUA). Left ventricular ejection fraction was calculated according to the modified Simpson's rule[15,16]. Holter monitoring was performed with a Dynamis 3000 ECO recorder and analyzed in a Burdick machine using AltairPC Holter System software.

Statistical analysis - Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows version 9.0. Continuous variables were presented as mean ± standard deviation. Variable distribution was assessed by the Kolmogorov-Smirnov test. Since variables were not normally distributed, non-parametric tests were used. All comparisons were two-tailed. P values < 0.05 were considered statistically significant.

RESULTS

Thirty patients were selected, but two were excluded after bone marrow collection: one patient due to coronary disease detected by catheterization and the other due to technical problems with the equipment. The primary characteristics of the 28 patients studied are described in Table 1.

An analysis of the clinical profile reveals a population with advanced stage of the disease and various markers of severity. All patients were extremely limited, in functional class III and IV, despite optimal treatment with a multi-drug regimen and high doses of diuretics. Functional capacity was found to be low, based on the short distance covered during the six-minute walking test, and quality-of-life was severely impaired, as indicated by high scores on the Minnesota questionnaire. Ventricular function was severely depressed, with very low ejection fraction and high left ventricular diastolic diameter on the echocardiogram. The presence of marked hyponatremia and renal dysfunction characterizes the severe condition of this population.

There were no complications directly related to either bone marrow aspiration or cell injection. Viability tests showed that 96 ± 6.5 % of the cells were viable. Neither significant changes in myocardial necrosis markers in 24 hours nor electrocardiographic changes suggestive of ischemia or infarction occurred.

In order to check if the myocardial implantation of stem cells was associated with arrhythmias, the

Table 1 - Clinical and laboratory characteristics of the patients

	(n = 28)
Age (years)	52.2 ± 9.9
Male (n)	24
NYHA class (n)	
III	25
IV	4
6 min walking test (m)	355 ± 136
LV ejection fraction (%)	20.1 ± 6.8
LVEDD (mm)	72.6 ± 8.9
QOL score	50.9 ± 11.7
Serum sodium (mEq/L)	131 ± 7.6
Serum urea (mg/dL)	69 ± 36.8
Creatinine (mg/dL)	1.4 ± 0.4
Digoxin (%)	95
Furosemide (%)	85
Hydrochlorothiazide (%)	35
ACEI or ARB (%)	85
Beta-blockers (%)	55

n = number of patients; FC = functional class (NYHA); QOL = Minnesota quality of life score; LVEDD = LV end-diastolic diameter; ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin II type I receptor blocker. Values expressed in mean ± standard deviation

arrhythmogenic profile was evaluated through the total number of ventricular extrasystoles in 24 hours, as well as their clustering and recurrence pattern. No significant change was found in the arrhythmogenic profile, despite a non-significant trend for increase in the number of ventricular premature beats in 24 hours (Figure 1), with no change in the number of ventricular tachycardia episodes (Figure 2).

There were three deaths during the two-month follow-up, all during the second month (patient 04, male, age 37, pulmonary hemorrhage and respiratory failure; patient 16, female, age 34, sudden death; patient 27, age 69, male, end-stage heart failure). Although this study was not designed to assess efficacy, and that mortality cannot be evaluated as a safety endpoint, except by the causal association of immediate temporariness, no direct causal association was found between death and cell transplantation. There were no neoplasias,

hematological diseases, coagulopathies or any other kind of disease that could be attributed to cell injections or their implantation.

Ventricular function, evaluated by left ventricular ejection fraction showed significant improvement one month after the procedure, which was maintained at two months (Figure 3).

Quality of life assessment by the Minnesota Living with Heart Failure questionnaire revealed significant improvement in the global score, also at one month and was maintained throughout the follow-up period (Figure 4).

Functional capacity, as measured by the distance walked during the six-minute corridor walk test, showed significant improvement, which was maintained for two months (Figure 5).

When functional class was analyzed as an ordinal variable, a decrease in mean NYHA functional class was
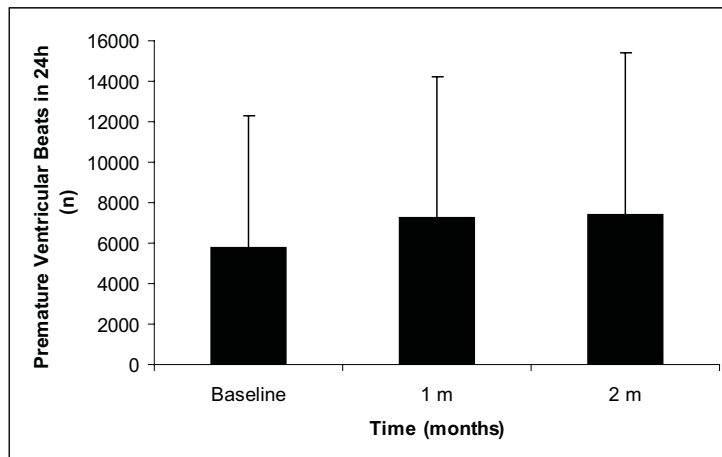

Fig. 1 - Non-significant trend of elevation in the number of ventricular premature beats in 24 hours. VE = ventricular extrasystoles p = 0.062 (Friedman). Bars represent mean ± standard deviation.
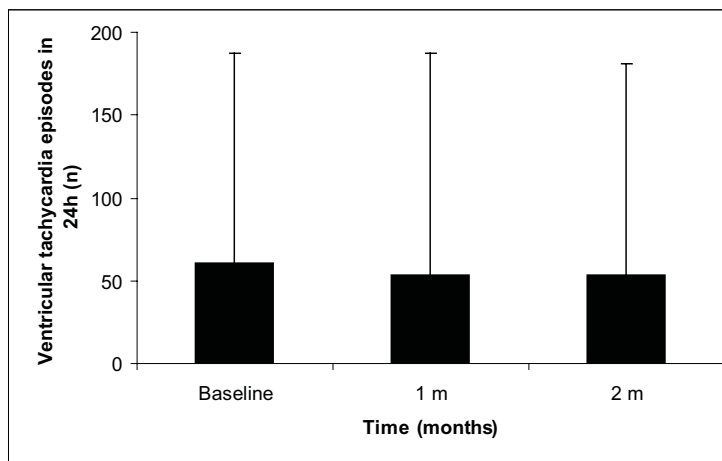

Fig. 2 - No elevation in the number of ventricular tachycardia episodes in 24 hours. T = ventricular tachycardia p = NS (Friedman). Bars represent mean ± standard deviation.

noted after one month and maintained over the follow-up period (Figure 6).

Biochemical analysis showed correction of serum sodium concentration between 72 hours and three weeks of treatment, which was maintained over the first two-months of study follow-up (figure 7).

DISCUSSION

This is the first study to report the implantation of bone marrow mononuclear cells in patients with heart failure due to Chagas disease and the first cell therapy study using a non-ischemic model. Our model involved intracoronary administration of stem cells, followed by bone marrow stimulation with subcutaneous filgrastim after 30 days.

Patients in this study are more severe than those previously reported in other stem-cell studies of ischemic etiology. In the other studies, patients had milder degrees of ventricular dysfunction, renal dysfunction and hyponatremia. The number of injected cells in the present study was greater than that of previously published studies. Whether there is a relationship between the number of cells injected and the degree of clinical response to cell therapy remains unclear, but this hypothesis warrants further investigation.

The absence of adverse events directly related to the procedure indicates that this is feasible, provided that the protocols implemented in this study are followed, as they aim at the patient's protection and safety.

One of the most relevant aspects analyzed regarding safety was whether bone marrow cell injection into the coronary system could trigger acute myocardial infatction. To ascertain this, troponin I and CK-MB measurements were performed, as well as serial electrocardiograms, which showed no evidence of
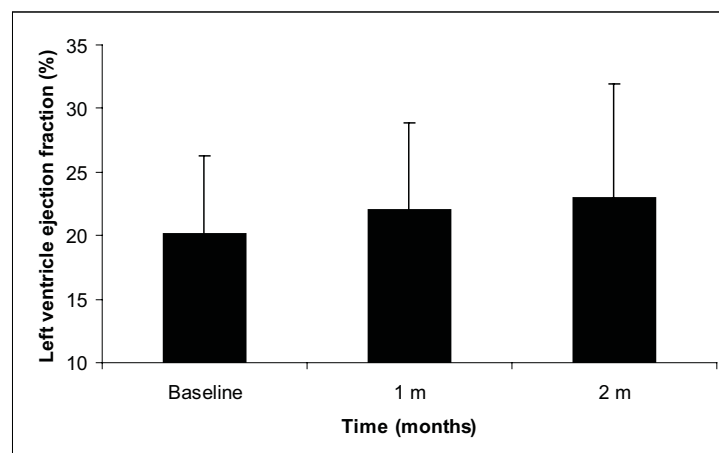

Fig. 3 - Left ventricular ejection fraction by radionuclide ventriculography. An increase is observed after 30 days and maintained at 2 months. p = 0.02 (Friedman).
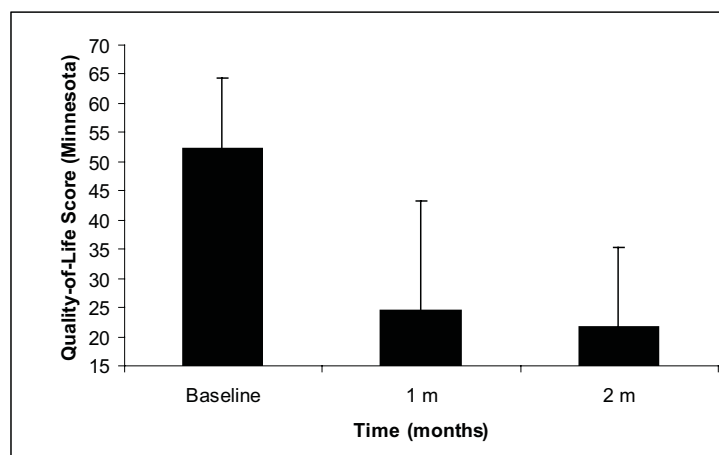

Fig. 4 - Quality of life score (Minnesota questionnaire). A significant decrease in the score is noted, indicating improvement in quality of life. p < 0.0001 (Friedman).
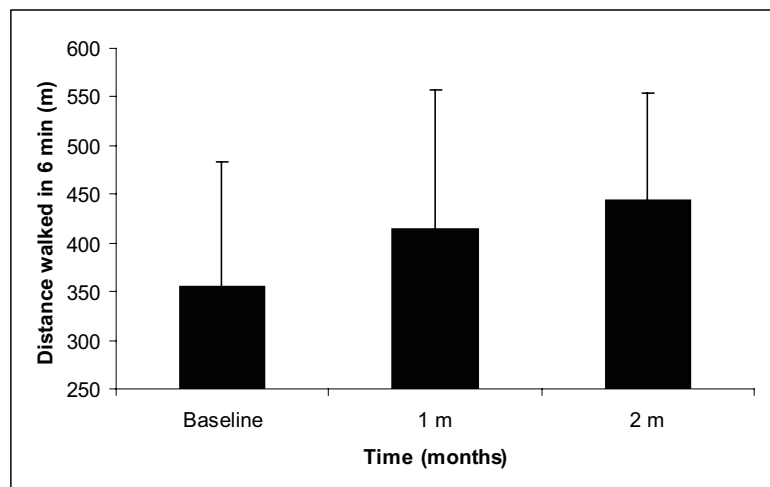
Fig. 5 - *Distance covered in the six-minute corridor walk test. A significant increase is noted after 30 days and maintained at 2 months. p = 0.003 (Friedman).*

myocardial injury, thus suggesting that this procedure is safe regarding ischemia inducement.

Another important parameter analyzed was the potential to induce arrhythmias. Previous studies using skeletal muscle stem cells (satellite cells) have shown promising results, with partial recovery of cardiac function; yet, there has been concern regarding the development of arrhythmias in patients that underwent this kind of cell therapy[17,18]. In our study, the number of isolated ventricular extrasystoles increased significantly, but the number of sustained or nonsustained ventricular tachycardia episodes remained unchanged.

As far as parameters related to efficacy are concerned, LVEF increments were similar to those described in previous studies. In the BOOST study[19], mean LVEF was 52%, and the authors detected an increase of 5%, on average, corresponding to around 10% of relative increase. In the present study, mean LVEF increase was similar; yet, it corresponds to approximately 20% of relative increase, since LVEF of our patients was significantly lower.

One finding that calls attention is the improvement in quality of life and functional capacity. Despite being on optimal treatment and multi-drug regimen in optimal doses, patients' functional capacity and quality of life remained severely impaired. However, their quality of life improved consistently, as evaluated by the Minnesota questionnaire, which reflects not only dyspnea parameters, but also emotional, psychological, economic, and professional aspects. The same was true with respect to more objective variables, as distance covered during the six-minute walk test, which measures exercise capacity at submaximal levels. Consistent with the variations mentioned above, there was a significant improvement in functional capacity, as assessed by the NYHA classification. While motivational aspects may

be related to these subjective variables, the consistent modification suggests that an underlying clinical effect is likely to exist. All these findings were observed only one month after treatment, too early to be considered an effect of transdifferentiation or cell fusion. In that regard, recent evidence indicates that the mechanism of action associated with stem cell transplantation may involve three different phenomena: transdifferentiation, cell fusion, and paracrine effects. This hypothesis is based on the observation that measurable effects may be demonstrated as early as 72 hours[20].

More objective variables, irrespective of motivation or self-suggestion by the patient, as well as investigator interference, indicate that the therapy used produced positive biological effects. Hyponatremia, for example, is one of the most important prognostic markers in advanced CHF, and its pathophysiological mechanism is related to the neurohumoral activation of the syndrome[21]. The hyponatremia correction achieved within two weeks in this study is uncommon in clinical practice, where it is hardly ever observed in severely ill patients, even after optimal therapeutic interventions.

Overall, the above-mentioned results open new perspectives for the treatment of heart diseases through the regenerating capacity of stem cells. Our data suggest that transplantation of bone marrow mononuclear cells to the myocardium of patients with heart failure due to Chagas disease is feasible, potentially safe, and effective, bringing symptomatic relief to and improving the quality of life of a significant portion of the Brazilian population affected by Chagas disease. On the other hand, further studies focused on elucidating the mechanisms leading to improvement after therapy are clearly needed, as well as establishing more effective protocols and determining potential risks and limitations associated with the methodologies used. While the accurate identification
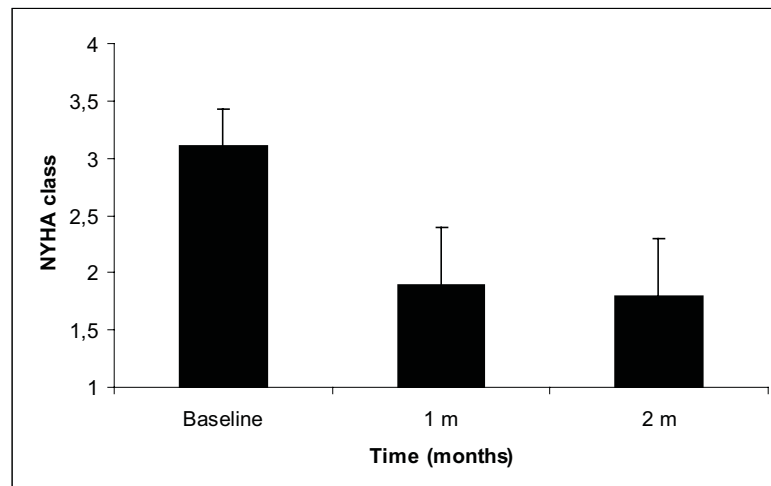
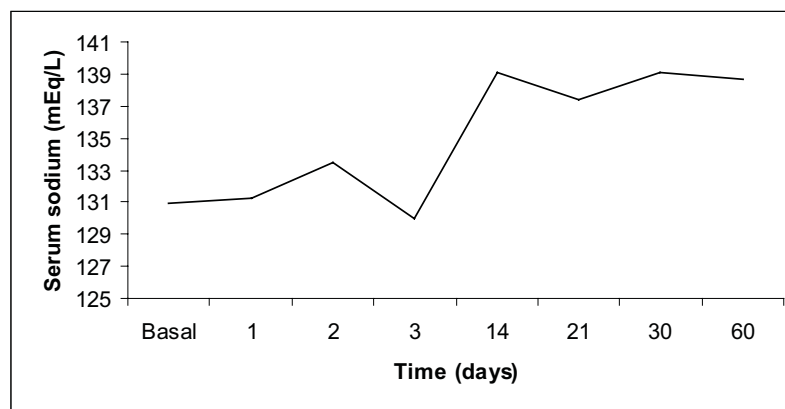**Fig. 6** - *Mean functional class (NYHA) before and after treatment. p < 0.0001 (Friedman).*

Fig. 7 - *Serum sodium concentration before and after treatment. Sodium concentration was normalized between 72 hours and 2 weeks and maintained up to study conclusion (Friedman, p < 0.0001).*

of subpopulations involved in the repair process is an important issue that warrants investigation, it is essential to evaluate cautiously whether other methods for purifying these subpopulations, indeed more expensive than the simple purification of the mononuclear fraction used today in clinical trials, will bring more benefit to patients.

The corroboration of our findings in a longer-term follow-up, as well as in randomized controlled clinical trials, may provide a new therapeutic line available worldwide at a very low cost and with no rejection, requiring no specific medication and that is more physiological, less aggressive and allows multiple repetitions.

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Acknowledgement

Potencial Conflict of Interest

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

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