



Research Article

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## Significant Improvement in Ejection Fraction and Functional Class after Two Years of Cells Transplantation

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### Abstract

To mitigate the Idiopathic dilated cardiomyopathy in the world, about 16,000 transplants of autologous stem cell allogeneic and 29 000 are performed. In Brazil, the transplantation of autologous bone marrow stems cells increased by about the 1000 per year. The aim of this study was to identify short-term results by intracoronary infusion of autologous mononuclear layer cells from bone marrow by SEPAX in patients with idiopathic dilated cardiomyopathy to examine improvement in Functional Class and Ventricular Ejection Fraction. In the methodology, of the 15 patients with a mean age of 59 years, nine were male and six were carriers of resynchronization. Thus, access to the infusion of stem cells from mononuclear layer was the femoral artery and the coronary arteries were selected angioplasty guide catheters. In flow rate of 1.0 mL per minute, were infused 10.0 mL of cell solution in AD, 2.0 mL in LxC and 8.0 mL in RCA when it was dominant, and in cases of left dominance, was infused 8.0 mL in LxC and 2.0 mL in RCA. As a result, of the 15 patients before treatment, 10 had Functional Class IV and Functional Class III and five demonstrated after treatment, 1 patient was in class I, 11 patients were in Functional Class II and 3 patients were in Functional Class III. Have Ventricular Ejection Fraction before treatment averaged 23.6% and post - treatment average was 36.27%. There was no significant difference between patients with and without pacemaker. After treatment with pacemaker the Ventricular Ejection Fraction was 37.33 and Ventricular Ejection Fraction 35.48% without pacemaker. In conclusion, treatment with intracoronary infusion of stem cells from mononuclear layer showed significant improvement in Functional Class and Ventricular Ejection Fraction in patients with Idiopathic dilated cardiomyopathy, refractory to therapy, over a period of 12 to 24 months after treatment.

### Keywords

Stem cells from mononuclear layer; Bone marrow; Telocytes; Cardiomyopathy; Cell therapy

### Introduction

Idiopathic dilated cardiomyopathy (IDC) is a major public health problems in the Western world. In the United States, approximately

5.7 million people suffer from congestive heart failure, according to American Heart Association [1,2]. In addition, patients with IDC in functional class IV have high mortality. To mitigate this problem in the world, are held about 16,000 transplants of autologous stem cells and 29,000 allogeneic [1,3]. In Brazil, the transplantation of autologous stem cells from bone marrow increases about 1000 cases per year [4].

Hospitalization costs worldwide exceed \$ 1 billion annually, quality of life is poor, and thousands of lives are lost each year [5]. In Brazil, it is estimated that 2.0% of the population are affected by dilated cardiomyopathy and cardiovascular mortality rate in 2004 was 286 people per 100,000 inhabitants, and the forecast for 2020 of 400 people per 100,000 populations [4].

Dilated cardiomyopathy is a disease with few therapeutic options, and very often the organ transplantation [5,6,13], and most patients on the waiting list die before receiving a new heart. Therefore, a therapy it is necessary to be able to improve heart function and more accessible to the population is of great interest. Thus, cell therapy with bone marrow stem cells have shown promising results in the literature as improvement of life of patients with IDC [1,8,12]. Thus, studies in ischemic heart disease demonstrated that stem cells obtained from various sources, including bone marrow may contribute to cardiac regeneration, also indicating the potential use of stem cell therapy in other cardiomyopathies [4,21].

Thus, hematopoietic and mesenchymal stem cells from bone marrow through paracrine effects, autocrine and endocrine and together with other cells as telocytes [15,18-21], cardiomyocytes and macrophages can promote cardiac tissue regeneration and neovascularization in the heart [2,3]. Thus, the cardiac function can be improved with an average 23% increase in the ejection fraction to 43% and also functional class improved several clinical trials according to the American Heart Association [4,10]. However, there are many flaws of standardized responses to the cell therapy efficiency worldwide, being necessary to increase the randomized multicenter studies, making it clear that there is an information gap cell therapy.

Thus, some literary findings corroborate the information gap was investigated in patients with isolated atrial amyloidosis (IAA) the ultrastructure of cardiomyocytes and telocytes in patients with atrial fibrillation (AF) and IAA, with contradictory findings to the interest. Human atrial biopsies were obtained from 37 patients undergoing cardiac surgery, and AF 23 (62%). The microscopic examination electronic (ME) has certified that 17 patients had IAA and 82% had AF. Still ME showed that these deposits are, in fact, located at interstitial recesses. In addition, revealed that telopodes (present in telocytes) surrounding the amyloid deposits by limiting its spread into the interstitium. Thus, the findings revealed that the particular connection telopodes with amyloid deposits suggests their involvement in isolated atrial amyloidosis and pathogenesis AF [21].

In addition, other published results showed a positive trend in VEF- ventricular ejection fraction by magnetic resonance imaging, 22.64% to 29.75% to 32.69% after the first and second year of stem cells for bone marrow transplant dilated cardiomyopathy, respectively, and statistically significant when compared with the

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standard pharmacological treatment. Moreover, cardiac cells and mesenchymal stem cells derived from CD117 can improve cardiac function following injury. However, no studies have compared the therapeutic benefit of these cells when used autologously [8,17-19].

These results were similar to those for chronic myocardial ischemia in a multicenter randomized study [31,32]. In 2007, 17 studies are available that assessed the changes in left ventricular function after cell therapy, most of them indicated small improvements in VEF (ranging from 2.9% to 9.3%) [36], we found an improvement in VEF 7.11% after the first year and 10.05% after the second year of cell therapy, probably the described literature.

The objective of this study was to identify short-term results by intracoronary infusion of autologous mononuclear stem cells in patients with IDC to analyze improvement of CF and Ventricular Ejection Fraction (VEF).

## Materials and Methods

### Materials

The materials used were trypan blue (Sigma Aldrich, St. Louis, MO, USA), Kit monoclonal antibody CD34 (+), CD34(-), CD 133(+), CD 133(-) and immunohistochemistry with positive reactions for c-kit, vimentin and CD34 for Telocytes (BD Biosciences, South America, USA), Kit SEPAX (Biosafe America, Inc.1225 North Loop West, Suite 120, Houston, TX 77008, USA), Flow Cytometry (BD Accuri C6™, BD Biosciences, South America, USA). As the density gradient Ficoll-Hypaque was used (Amershan Biosciences, Piscataway, NJ, USA).

### Methods

**Experimental design:** The study was blinded, randomized and performed at 15 patients with a mean age of 59 years, and six were carriers of resynchronization (RC). Bone marrow was processed by SEPAX automated system for the isolation of autologous mononuclear layer (AML) of 20 mL with a mean of  $3.0 \times 10^8$  total cells, the average being equal to  $2.0 \times 10^3$  leukocyte cell. After this, access to the infusion of autologous mononuclear layer cells from bone marrow was the femoral artery and the coronary arteries were selected angioplasty guide catheters. The guide wire was 0.014 sequentially positioned in the anterior descending artery (DA), left circumflex (CXE) and right coronary artery (RCA). Were infused in flow of 1.0 mL per minute, 10.0 mL of cell solution in AD, 2.0 mL and 8.0 mL in CXE the CD when it was dominant, and in cases of left dominance, infundia- to 8.0 mL and 2.0 mL in CXE the CD. In addition, catheterization was performed for the evaluation of ventricular function at 12 and 24 months. **The present study was approved by Brazilian National Research Ethics Committee (CONEP) (Registration #: 15342).**

The Randomized Assesment of Digoxin on Inhibitors of Angiotensin-Converting Enzyme (RADIANCE) trial found that discontinuing digoxin in patients with low ejection fraction and HF resulted in worsening HF, Current American Heart Assaociation/ American College of Cardiology guidelines classify digoxin use as IIA in patients with current or prior symptoms of HF and reduced left ventricular ejection to decrease hospitalizations for HF. B-Blockers should be maintained in this hemodynamically stable patient (Class I indication) because there is no evidence that routinely discontinuing  $\beta$ -blockers in this setting is beneficial. Patients such as this usually respond well to intravenous loop diuretics (avoiding the absorption issues of oral diuretics in the setting of intestinal edema),

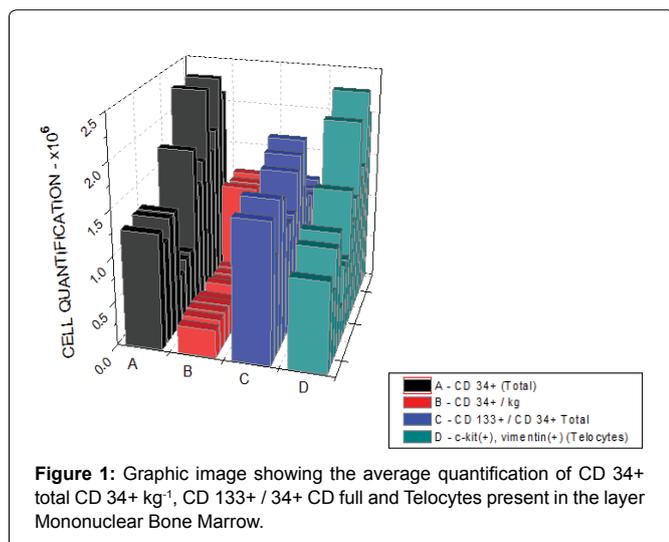
with improved renal function as preload is optimized. In patients who are true refractory to diuretics, intravenous nitroglycerine or ultrafiltration can be helpful (Class II).

**Bone marrow aspiration, isolation of mononuclear cells and cell Injection:** Before the arrival and manipulating bone marrow sample, it was “check list” materials and reagents required for handling Kit (SEPAX) and bone marrow, such as syringes, needles, 70% ethanol, gas, human albumin, ficoll and serum. Called up the equipment SEPAX, at least thirty minutes prior to stabilization of electric current. Sterilized room and laminar flow hood with UV light for fifteen minutes. The Kit was manipulated inside the laminar flow hood. The first step to manipulation of the kit was to examine whether all three “taps” were in the position “T”. The second step was to close all clamps. The third step was to inject 100 mL of Ficoll purse specific washing. The fourth step was to discard 62.5 mL serum bottle and inject the same volume of albumin and connect the bottle right output Kit. The fifth step is to connect the empty bag (for storing the mononuclear cell layer) and the sixth step in the Kit was connected the bag with the bone marrow. For handling the SEPAX was necessary to follow the instructions of the standard protocol for operating the machine in the category of “separation by ficoll.” After mounting, the verification was made of three settings: sample volume (60 mL), number of washes (two washes for 500 mL serum). After this, the “enter” checked the Kit after checking Kit, opened up all the clamps. Soon after, the software asked to do the pumping purse bone marrow and then tightened “up” twice to adjust the trajectory of the spinal cord to close the entrance. Thereafter, if pressed “enter” to start the process. First, the Ficoll was aspirated into the tube. Then, the marrow was slowly aspirated into the tube on top of the Ficoll. It started spinning and then the fraction of erythrocytes and granulocytes was discarded and mononuclear ficoll layer was aspirated and washed with saline. After one hour the process ends and the mononuclear cell layer was available for quantifying CD 34 (+), CD 133 (+), vimentine, c-kit after obtaining the pellet by centrifugation [28]. The final suspension of bone marrow mononuclear cells contained  $3.0 \times 10^8$  totals cells (being  $5.0 \times 10^6$  CD 34+ cells,  $2.5 \times 10^6$  CD 133+ cells,  $3.0 \times 10^6$  Telocytes and others cells as monocytes) was injected intracoronary in the affected area. The placebo group received intracoronary injection of saline plus autologous serum of the patient solution, to ensure that the study was double-blind, keeping the color of the injected solutions. Before every injection, the catheter was positioned perpendicular to the endocardium with excellent loop stability and the extension of the needle had to induce a premature ventricle contraction.

**Statistical analysis:** In this single-center, double-blind study compared the amount of infiltrated cells, the sample size was 15 patients, 06 patients with resynchronization. We conducted descriptive statistical analysis on the quantitative values of mononuclear bone marrow and telocytes and also the percentage of ejection fraction, obtaining means and standard deviations. In addition, the test was made of the Kolmogorov-Smirnov normality and Pearson correlation between the number of CD34 + cells and telocytes and also the test signal in order to analyze the degree of similarity between the samples. For studies of functional class and ejection fraction, became the Wilcoxon test. All results were obtained using the R and OriginPro 08 programs.

## Results

The quantification of cells present in bone marrow mononuclear layer and telocytes are shown in [Figure 1](#). According to the statistical



analysis of the test signals by means of the correlation between the related samples, the amount of total cell marker CD 34+ is directly proportional to the amount of telocytes because of the 15 samples analyzed, 13 were directly correlated quantity,  $p=0.004$ , with excellent critical level of significance.

Furthermore, by means of Figure 2, the results of significant improvements to the functional class change is also related to patients who received increasing amounts of CD34+ and Telocytes. Thus, of the 15 patients before treatment, 10 had class IV and five presented FC III and after treatment, 1 patient was to FC I, 11 patients were in FC II ( $p < 0.001$ ) and 3 patients were in FC III ( $p < 0.001$ ), according to figure 2. Have VEF before treatment had an average of 23.6% ( $\pm 3.81$ ) and after treatment the average was 36.27% ( $\pm 2.17$ ),  $p < 0.05=0.007$  with 95% confidence interval. There was no significant difference between patients with and without RC, and in the pre-treatment with RC VEF was 25.24% ( $\pm 3.91$ ) and 21.61% ( $\pm 1.17$ ) without RC. After treatment with RC VEF was 37.33 ( $\pm 2.6$ ) and VEF 35.48% ( $\pm 3.6$ ) without RC, according to figure 3.

All patients were followed every six months with all appropriate clinical tests to analyze cardiac functions standards, comparing them with previous clinical situations up to 2 years complete analysis of the patients.

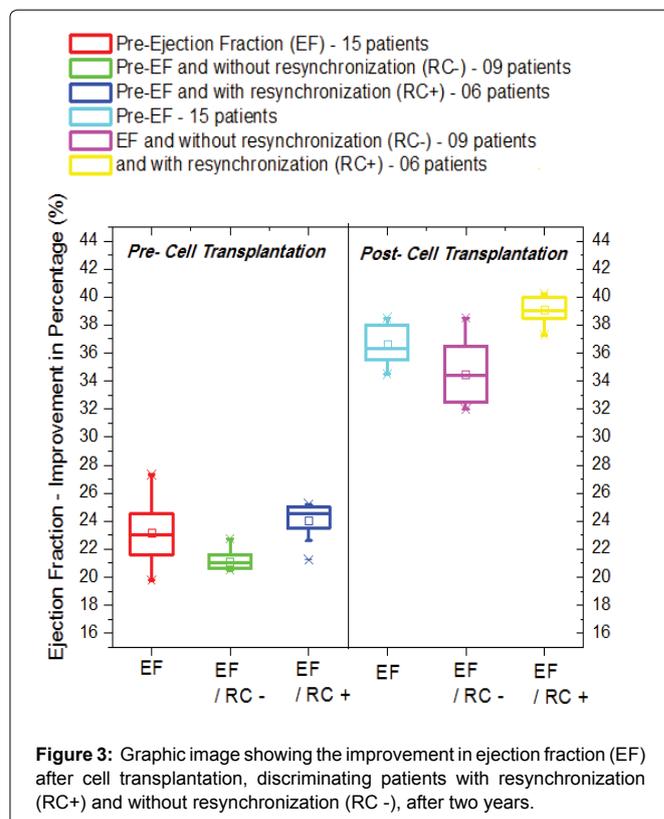
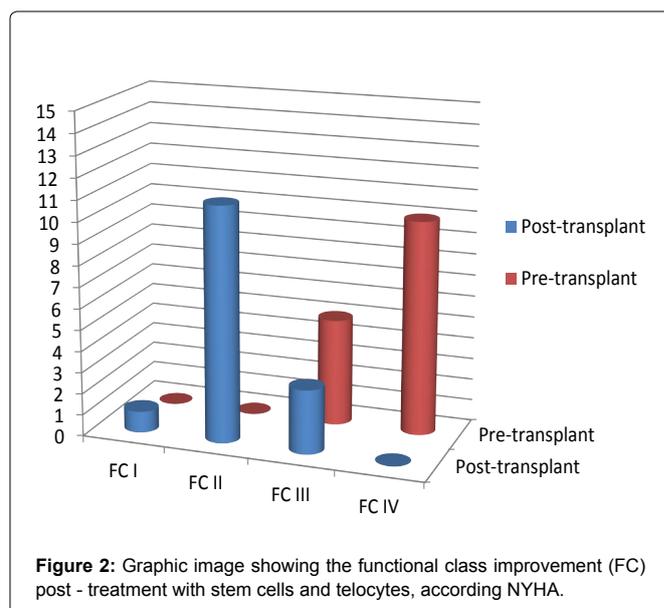
## Discussion

The effect of improvement of cardiac functions in tissue regeneration and angiogenesis may be due to paracrine effect (inflammation) caused by hematopoietic and mesenchymal stem cells from bone marrow, stimulating the process of "homing" of macrophages, neutrophils, monocytes, stem cells and telocytes, and through transdifferentiation and differentiation of cells infused [1,6,14]. In the case of a paracrine effect, the first highlighted are macrophages can be induced stem cells for the production of nitrogen oxide (NO), it is important to induce angiogenesis, vasodilation and as an inducer of differentiation of stem cells into cardiomyocytes. The second telocytes are highlighted, which play an important role in intercellular communication [6,22-24].

Added to this, the author Popescu et al. (2005) [6] showed the importance of the presence of Telocytes (CT)-Origin mesenquimal-with stem cells in increasing the efficiency of cell therapy. The

presence of CT has been documented in several organs and cavities in the connective tissue (heart, the pericardium, the pulmonary vein, intestine, mesentery, gallbladder, uterus and fallopian tubes and non-organ cavity [19,20].

In addition to the mechanical support function for the CT system, it is believed that the main role of CT is intercellular communication and control [20,21]. Thus, the CT location in close proximity to the blood capillaries or nerve endings. To deepen, several studies



have hypothesized that CT in their mechanical and intercellular signaling functions can guide immature cells during organogenesis, and precursors of mesenchymal cells in adulthood. It is therefore proposed that the TC can be extremely important in the regeneration of tissues and organs, although there is still no clinical evidence [6,21].

Therefore, the stem cells in quiescent state can proliferate rapidly when stimulated with growth factors released by stem cells infused, macrophages and telocytes [6,43]. Then, the inflammatory process became important to a certain stage (about 7 days) and, added to this is a suitable biological niche in the heart area that promoted the adaptation and differentiation of stem cells into the desired tissue [25,42]. It was suggested, so that after the adaptive phase the cells, there was an exponential growth of these cells, which covered the area of fibrosis of the heart with healthy heart gain after 2 years months of treatment [26-28].

To corroborate this study, pre-clinical study of left ventricular dysfunction after myocardial infarction in pigs undergoing infusion of stem cells from bone marrow, there was heart tissue regeneration, with hemodynamic improvement [16]. Another study examined the direct administration of mesenchymal stem cells from bone marrow in the left ventricular wall in co-culture with skeletal myoblasts for 14 days, with improvement in ejection fraction of 23% to 34% of heart, with contraction of the stroke volume, reduction in diastolic blood pressure and the occurrence of angiogenesis [9].

A preclinical study explored the myocardial infusion procedure for catheterization of mesenchymal stem cells in rats [39]. After 4 weeks, cardiac function was assessed by echocardiography. MRNA expression of type I infarction, collagen type III and transformation growth factor (TGF) - $\beta$ 1 were detected by RT-PCR. Heart function were improved. Thus, it is suggested that cell-based therapies are promising intervention for the treatment of heart failure (HF) secondary to ischemic and non-ischemic cardiomyopathy. However, the clinical effectiveness of new treatment requires evaluation.

So, was held in another study the clinical evaluation of the safety and efficacy of therapies based on stem cells and telocytes to heart failure (HF) [4]. We used electronic databases CENTRAL, daring, NHSEED & ATS, PubMed, MEDLINE, EMBASE, CINAHL, LILACS, KoreaMed, PakMediNet, IndMed and Transfusion Support Library to find randomized clinical trials to June 2014. Testing of participants with HF and wherein the administration of any dose of autologous cells by any route was compared to placebo were eligible for inclusion. The sample size was 1,521 participants. The treatment significantly reduced the risk of mortality and re-hospitalization for HF. There was significant improvement in performance and exercise capacity, left ventricular ejection fraction and quality of life. The treatment was also associated with a reduction in BNP levels and no increase in the incidence of arrhythmias [4].

In addition, other studies have shown that administration of positive cardiac c-kit-autologous stem cells in patients with ischemic heart failure led to impressive results [29-35]. On average left ventricular ejection fraction of cells treated group increased from 30% at baseline to 38% after one year and 42% after two years of cell injection. Some of them are related to the intrinsic property of stem cells residents, such as direct differentiation, paracrine action and immunomodulatory function [36-38]. In addition to this, it was shown that cardiac stem cells for therapeutic purposes can be prepared from small biopsy samples as well as frozen tissues, such

strategies against various cardiovascular diseases, including non-ischemic cardiomyopathy, congenital heart disease [5,40,41].

## Conclusion

In conclusion, treatment with intracoronary infusion of AML showed significant improvement in CF and VEF in patients with IDC, refractory to therapy in a period of 12 to 24 months after treatment. The presence of the resynchronization not showed to influence significant the outcome of treatment.

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## Approval of the Ethics Committee

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