Biodistribution of bone marrow mononuclear cells in chronic chagasic cardiomyopathy after intracoronary injection

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ABSTRACT

Background: Animal and human clinical studies have indicated that bone marrow (BM) mononuclear cell (MNC) therapy for Chagasic Cardiomyopathy (ChC) is feasible, safe and potentially efficacious. Nevertheless, little is known about the retention of these cells after intracoronary (IC) infusion.
Methods: Our study investigated the homing of technetium-99m (^{99m}Tc) labeled BM MNCs and compared it to thallium-201 (^{201}Tl) myocardial perfusion images using the standard 17-segment model. Six patients with congestive heart failure of chagasic etiology were included.
Results: Scintigraphic images revealed an uptake of $5.4\% \pm 1.7$, $4.3\% \pm 1.5$ and $2.3\% \pm 0.6$ of the total infused radioactivity in the heart after 1, 3 and 24 h, respectively. The remaining activity was distributed mainly to the liver and spleen. Of 102 segments analyzed, homing took place in 36%. Segments with perfusion had greater homing (58.6%) than those with decreased or no perfusion (6.8%), $p < 0.0001$. There was no correlation between the number of injected cells and the number of segments with homing for each patient ($r = -0.172$, $p = 0.774$).
Conclusions: These results indicate that ^{99m}Tc-BM MNCs delivered by IC injection homed to the chagasic myocardium. However, cell biodistribution was heterogeneous and limited, being strongly associated with the myocardial perfusion pattern at rest. These initial data suggest that the IC route may present limitations in chagasic patients and that alternative routes of cell administration may be necessary.

1. Introduction

Chronic Chagas disease is a consequence of infection by the protozoan parasite *Trypanosoma cruzi* and is endemic in certain regions of Latin America. Recent estimates from the World Health Organization indicate that 18 million persons are chronically infected and 200.000 new cases occur each year [1]. Chronic cardiomyopathy is the most important and severe manifestation of human Chagas disease, affecting approximately 30% of infected patients [2,3]. Patients with end-stage Chagas disease have long been recognized to have a low survival rate and presently there are no effective therapies other than heart transplantation [2]. In this setting, the use of cell therapies may offer an alternative for these patients. Experimental models in mice with ChC have suggested BM MNC therapy has beneficial effects [4,5] and human clinical studies have indicated that BM MNC therapy is feasible, safe and potentially efficacious [6]. Nevertheless, little is known about the homing, retention and mechanisms of action of these cells. In ChC, myocardial perfusion defects, fixed or reversible, have been described and attributed to microvascular disease and fibrosis [7–10]. These perfusion abnormalities could affect the biodistribution of BM MNCs after IC injection, limiting their homing and their potential benefits. Therefore, using labeled cells we investigated the biodistribution pattern of BM MNCs after IC injection in chagasic patients, and compared it to ^{201}Tl myocardial perfusion scintigraphy.

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2. Materials and methods

2.1. Patients

Six patients with congestive heart failure of chagasic etiology that remained in New York Heart Association class III despite optimized treatment were included in this study. The general characteristics of the patients are described in Table 1. They had two positive anti-trypanosoma cruzi serological reactions by three distinct techniques (indirect hemaglutination, indirect immunofluorescence and enzyme linked immunosorbent assay). Uni and bidimensional Doppler echocardiographic analysis were performed in all patients prior to cell therapy. Echocardiographic views were obtained as recommended by the American Society of Echocardiography (ASE) [11]. Left ventricular systolic function was evaluated by the ejection fraction, estimated by Simpson's method [11]. The coronariography was normal in all patients. The study was conducted according to the guidelines of the local ethics committee and of the National Research Ethics Committee, and informed consent was obtained from all patients.

2.2. Cell harvesting, labeling and injection

Cells were obtained by marrow aspiration of the posterior iliac crest (50–80 ml) and processed by Ficoll density centrifugation at $400 \times g$ for 30 min (Ficoll 1.077, 1:2, Amersham Biosciences). After washing, cells were resuspended in 20 ml of saline solution with 5% autologous serum. Five ml (25%) of this solution was used for labeling with ^{99m}Tc as previously described [12–18]. In short, a saline solution of SnCl2 was added to the cell suspension in 0.9% NaCl and the mixture incubated at room temperature for 10 min. Then, 45 mCi ^{99m}Tc were added and the incubation continued for another 10 min. After centrifugation ($500 \times g$ for 5 min), the supernatant was removed and the cells were washed again in saline solution. The pellet was resuspended in saline solution.

Labeling efficiency was always >90%. Cell-bound activity ranged from 148 to 444 MBq (4–12 mCi). ^{99m}Tc-labeled cells demonstrated viability >95% as assessed by the trypan blue exclusion test. Cells labeled as described above were added back to the total mononuclear cell suspension (final volume of 20 ml) and slowly injected into the left (50%), right (25%) and left circumflex (25%) arteries, via an angioplasty catheter.

2.3. Cell characterization

A small aliquot of the mononuclear cells was used for immunophenotipic characterization of the injected cell population. Flow cytometry analysis was performed on a BD FacsCanto (BD Biosciences, USA) using antibodies (CD34, CD45, CD73, CD90, CD105, CD133 — all from BD Biosciences) that allowed identification of hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs). The injected mononuclear fraction contained a mean of 1.56% (range 0.9 to 3.30) HSCs (Ishage Protocol), 0.01% (range 0.003 to 0.011) MSCs and 0.01% EPCs (range 0.003 to 0.013).

2.4. Myocardial imaging

Myocardial ^{201}Tl and ^{99m}Tc-BM MNC images were acquired with a dual-head gamma camera (Millennium MG GE Medical Systems, Milwaukee, WI). ^{201}Tl myocardial perfusion Single Photon Emission Computed Tomography (SPECT) was performed at rest prior to IC cell therapy [19]. ^{99m}Tc-BM MNC planar and SPECT images were obtained 1, 3 and 24 h after IC infusion in all patients. Uptake was defined as the percentage of myocardium-originated number of counts, compared to the total number of counts of radiolabeled cells [19]. Short-axis, horizontal long-axis and vertical long-axis views for ^{201}Tl and ^{99m}Tc-BM MNC SPECT were obtained for each patient [20,21]. Perfusion and cell homing data were also transferred to polar maps of 17 segments for each patient [20,21]. The image analysis was performed by 2 independent observers, without knowledge of clinical data.

2.5. Statistical analysis

Interobserver agreement to define the segmental biodistribution of labeled cells and perfusion with ^{201}Tl was evaluated using the Kappa test. The chi-square test was used to compare the proportion of cell homing in segments with and without perfusion. The significance level adopted was 5%. After evaluating the pattern of distribution by the Kolmogorov–Smirnov Test, the correlation between the number of segments with homing and the total number of injected cells, as well as the number of CD34+, MSC and EPC cell populations, was analyzed by Pearson's product-moment correlation coefficient.

3. Results

The general characteristics of the patients, as well as the number of cells injected in each patient are shown in Table 1. Scintigraphic images revealed an uptake of 5.4% ± 1.7, 4.3% ± 1.5 and 2.3% ± 0.6 of the total infused radioactivity in the heart after 1, 3 and 24 h, respectively. The remaining activity was distributed mainly to the liver, spleen, kidneys and bladder in all time points. Intestinal uptake was observed 24 h after IC infusion. Fig. 1 illustrates whole body planar views of a representative patient. ^{201}Tl SPECT and ^{99m}Tc-BM MNC SPECT images allowed us to compare perfusion and cell homing in all patients. Long-, vertical- and short-axis views of the myocardium revealed a decreased uptake of ^{201}Tl in inferior, inferolateral and apical walls in all patients, corresponding to the areas of hypokinesis on the echocardiograms. ^{99m}Tc-BM MNC SPECT indicated a very heterogeneous uptake, but for all patients there was lack of or decreased uptake of ^{99m}Tc-BM MNC in walls with perfusion defects. Fig. 2 shows this comparison for two representative patients. Fig. 3 illustrates Polar maps for ^{201}Tl and ^{99m}Tc-BM MNC of a representative patient.

Furthermore, we performed a segmental analysis in all patients comparing perfusion and cell homing. In the ^{201}Tl study perfusion defects were identified in 44 (43%) of 102 segments analyzed. The perfusion pattern varied according to the myocardial segment (Fig. 4A). Taken together, the analysis of all 6 patients indicated that perfusion defects were more frequent in segments 4 (basal inferior), 5 (basal inferolateral), 10 (mid inferior), 15 (apical inferior) and 17 (apical). The study of cardiac biodistribution of the ^{99m}Tc BM MNC showed cell homing in only 37 (36%) of 102 segments analyzed. Cell homing varied according to the myocardial segment (Fig. 4B). Lack of homing was more frequent in segments 1 (basal anterior), 4 (basal inferior), 5 (basal inferolateral), 10 (mid inferior), 15 (apical inferior).

The analysis of the myocardial perfusion pattern and cell homing for each patient is shown in Fig. 5. For each patient the number of segments with or without perfusion and with or without cell homing was quantified and plotted in Fig. 5A and B, respectively. There was a strong association between perfusion and cell homing. Of 58 segments with perfusion, homing took place in 34 (58.6%) Of 44 segments without perfusion, homing took place in only 3 (6.8%), $p<0.0001$ as seen in Fig. 6. The analysis of interobserver variability showed a kappa of 0.529 ($p=0.000$) for perfusion and 0.455 ($p=0.000$) for cell homing. Also, the correlation coefficient between the number of injected cells and the
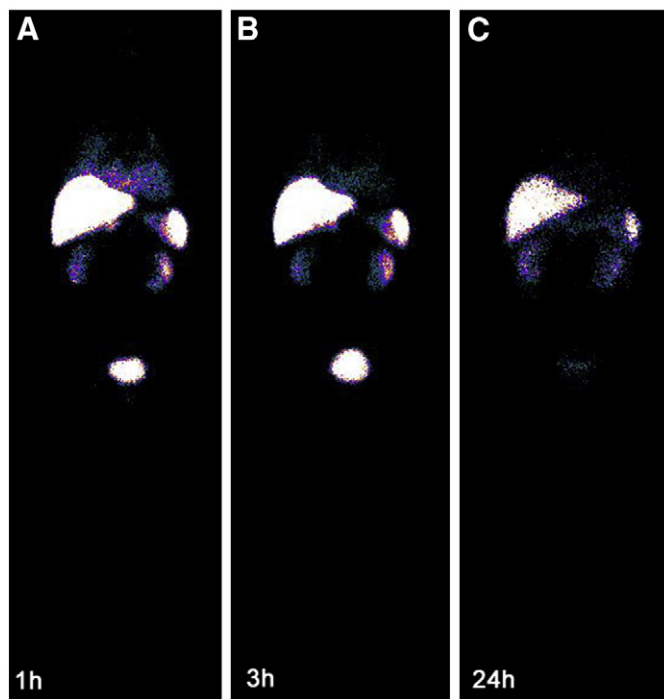
Table 1
Patients characteristics.

Patient	Age (years)	Gender	NYHA Class	LVEF at baseline (%)	Number of injected cells ($\times 10^8$)
1	53	M	III	27	5
2	56	F	III	29	9.5
3	62	M	III	32	9.6
4	59	M	III	23	1
5	49	M	III	28	3.6
6	60	M	III	24	2

Abbreviations: M = male; F = female; LVEF=Left Ventricular Ejection Fraction by Echocardiogram.

Fig. 1. Anterior views of whole body scintigraphy of a representative patient (Patient 1) carried out 1 (A), 3 (B) and 24 h (C) after cell infusion in the coronary arteries show the biodistribution of BM MNCs labeled with ^{99m}Tc. Black, no uptake; blue-red-yellow-white, increasing uptake.

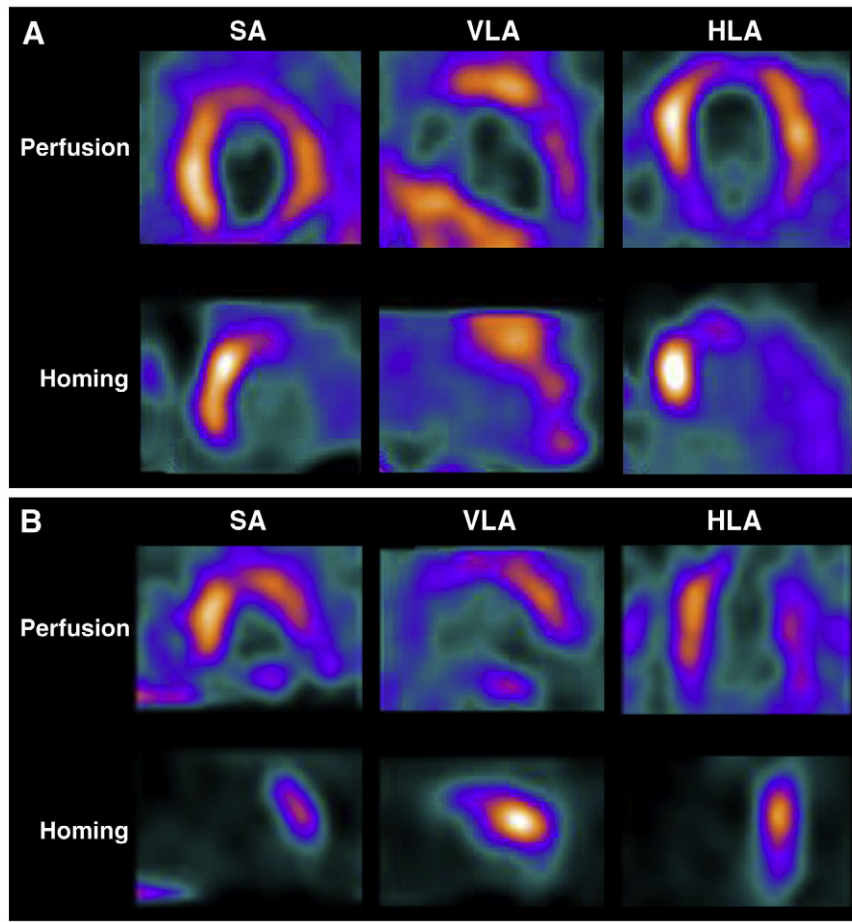**Fig. 2.** Comparison of ^{201}Tl SPECT demonstrating perfusion and ^{99m}Tc-BM MNC SPECT demonstrating homing of two representative patients (A — patient 2, B — patient 3) carried out 1 hour after cell infusion in the coronary arteries. SA=Short Axis; VLA=Vertical Long Axis; HLA=Horizontal Long Axis. Black, no uptake; blue-red-yellow-white, increasing uptake.

number of segments with homing was low or even negative ($r = -0.172$), with a nonsignificant p value ($p = 0.774$), as seen in Fig. 7. Likewise, correlation coefficients between the number of HSCs (CD34$^+$), MSCs (CD34$^-$/CD45$^-$/CD73$^+$/CD90$^+$/CD105$^+$) or EPCs (CD34$^+$/CD45$^-$/CD133$^+$) and the number of segments with homing did not reach statistical significance.

4. Discussion

Experimental models involving the injection of bone marrow derived cells in cardiac diseases have focused mainly in ischemic models and demonstrated an appreciable improvement in myocardial function that has recently been attributed to the cardioprotective or proangiogenic effects of secreted paracrine factors [22,23]. In humans,

the available evidence suggests that therapy with BM MNCs seems safe and associated with modest improvements in anatomic and physiologic parameters in patients with both acute myocardial infarction and chronic ischemic heart disease, above and beyond conventional therapy [24–30].

Chagasic cardiomyopathy is not a regional disease of the heart as are the ischemic syndromes. It affects the heart globally and it is characterized by an intense inflammatory infiltrate and fibrosis. In ChC, experimental models have been developed in mice and BM MNCs from control or *Trypanosoma cruzi* chronically infected mice have been used significantly decreasing inflammation and fibrosis for as long as 6 months after cell transplantation [4]. Another study using magnetic resonance imaging indicated BM MNC therapy resulted in a
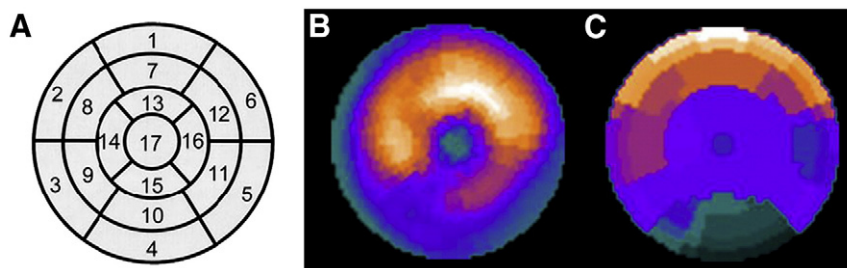

Fig. 3. Display, on a circumferential polar plot, of the 17 myocardial segments — 1: basal anterior; 2: basal anteroseptal; 3: basal inferoseptal; 4: basal inferior; 5: basal inferolateral; 6: basal anterolateral; 7:mid anterior; 8: mid anteroseptal; 9: mid inferoseptal; 10: mid inferior; 11: mid inferolateral; 12: mid anterolateral; 13: apical anterior; 14: apical septal; 15: apical inferior; 16: apical lateral; 17: apex (A). Polar maps of ^{201}Tl SPECT (B) and ^{99m}Tc-BM MNC SPECT (C) from a representative patient (Patient 5). Black, no uptake; blue-red-yellow-white, increasing uptake.
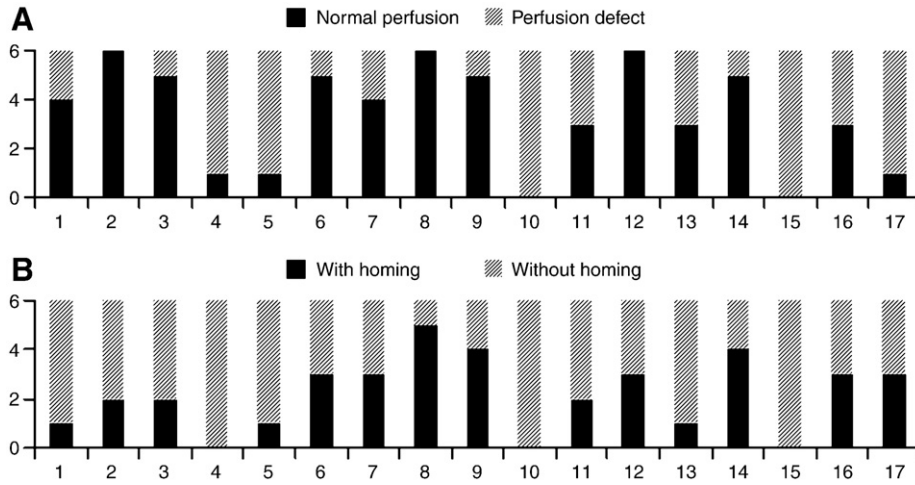
Fig. 4. Analysis of myocardial perfusion (A) and of cell homing (B) for each of the 17 myocardial segments.

significant reduction of the right ventricular dilatation typically observed in the chagasic mouse model [5]. In humans, a phase I clinical trial demonstrated that BM MNC IC cell injection in patients with ChC is feasible, safe and potentially efficacious, as recently reported [6]. Nonetheless, many questions remain unanswered about the homing, retention and possible mechanisms by which cell therapy may act in this disease [31].

In the present study we begin to address some of these questions by labeling cells with ^{99m}Tc to track them and investigate their homing and short time retention in the chagasic myocardium. Over the past fifteen years, we have developed and used a simple technique for the labeling of mononuclear leukocytes with ^{99m}Tc. It has been used for visualization of infection, osteomyelitis, graft rejection and fever of unknown origin [11–14]. When compared to techniques such as ^{99m}Tc-hexamethyl propylene-amine oxime (^{99m}Tc-HMPAO) labeling, there are advantages such as smaller amount of blood required, shorter performing time and lower cost [12–15]. Based on this experience, we have developed a new and simple technique for labeling BM MNCs and BM MSCs with ^{99m}Tc [16–18]. The 6-hour half-life of ^{99m}Tc allows the monitoring of cell distribution for approximately 24 h, which is an important advantage over the half-life of 110 min of 18F-fluorodeoxyglycose (FDG). In comparison with indium-111 oxine, another commonly used radio-pharmaceutical, ^{99m}Tc results in better image resolution and a lower radiation burden to the patient [12–15,32].

A case report had previously indicated cardiac homing of ^{99m}Tc-HMPAO labeled BM MNCs 2 h after their injection in one patient with ChC [33]. However, since ^{99m}Tc HMPAO-labeled cells were administered only into the anterior descending artery [33], this could have accounted for a
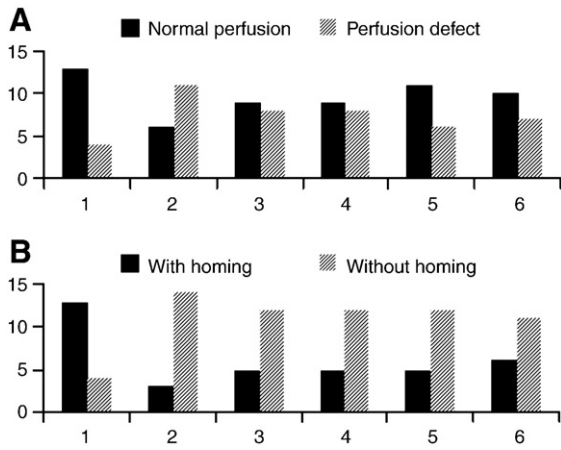

Fig. 5. Analysis of the myocardial perfusion pattern (A) and analysis of cellular homing (B) of each patient.
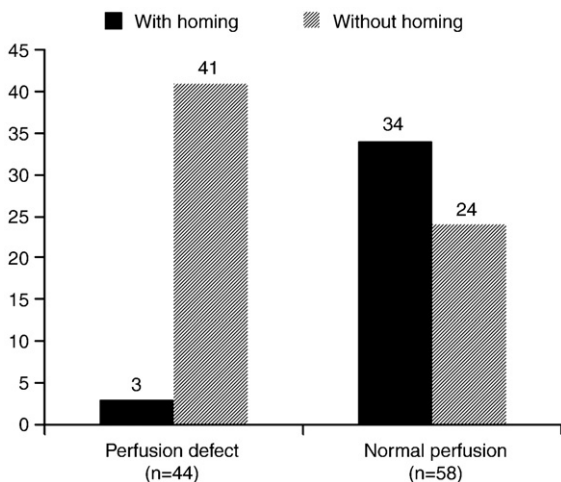

Fig. 6. Cell homing according to the myocardial perfusion pattern of all segments of the 6 patients. There was a strong association between perfusion and cell homing.
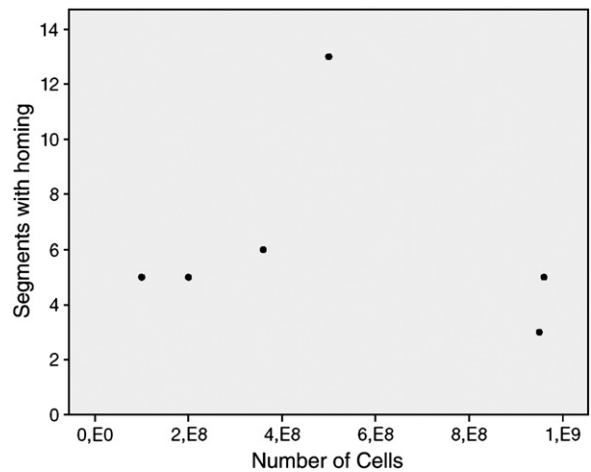

Fig. 7. Correlation between the number of injected cells and the number of segments with homing analyzed by Pearson's product-moment correlation coefficient.

limitation in the homing of BM MNCs. In our protocol, the labeled cells were injected into the three main arteries, in accordance to clinical trials involving IC BM MNC injection for ChC that don't evaluate cell homing [6,34]. In addition to the quantification of labeled cells, an analysis using the 17-segment model allowed a more adequate comparison between cell homing and myocardial perfusion.

In our study, cell homing was detectable in the heart up to 24 h after IC delivery, which is the longest possible time interval to track cells labeled by ^{99m}Tc. However, myocardial homing was found to be heterogeneous and limited. All patients presented extensive perfusion defects mainly in inferior, inferolateral and apical myocardial walls, where cell homing was absent or reduced, despite adequate injection in the corresponding coronary arteries. These perfusion deficits are consistent with previous reports on ChC and have been attributed to the presence of fibrosis and microvascular disease, already demonstrated in nuclear medicine, echocardiographic and anatomic pathology studies [7–10]. It is possible that these perfusion abnormalities may restrict cell homing, and this could represent a limitation to IC administration of BM MNCs in chagasic patients with greater perfusion defects, as the ones in this study. On the other hand, chagasic patients with less perfusion defects may have greater homing to the myocardium after IC injection. As for patients with more extensive perfusion defects, it is possible that other approaches may be necessary, such as intramyocardial (IM) injections. There are no studies comparing different techniques in ChC, but recent reports in experimental myocardial infarction models with peripheral blood MNCs [35], BM MSCs [36] and BM MNCs [37] have suggested that IM route may achieve higher myocardial retention rates when compared to IC infusion.

Our study provides for the first time quantification of BM MNC retention for up to 24 h after IC injection and indicates that cell homing may be decreased in myocardial regions with perfusion deficits in the setting of Chronic Chagasic Cardiomyopathy. These initial data suggest that the IC route may present limitations in chagasic patients and that alternative routes of cell administration may be necessary. Nevertheless, further experimental and clinical studies are warranted and, to this end, the field of nuclear cardiology may play an important role in the evaluation of parameters such as cell tracking, cell biodistribution and its correlation to myocardial perfusion/viability in Chagas disease.

Acknowledgements

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