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MEASUREMENT AND FEASIBILITY OF HEMATOPOIETIC STEM CELL WAS GREATER FOR EQUIPMENT IN CLOSED SYSTEM

[1,2,*]Idiberto José Zotarelli Filho, [2]Ana Carolina de Abreu, [2]Oswaldo Tadeu Greco, [2]Aldemir Bilaqui, Elias Naim Kassis, José Dalmo de Araújo, José Luiz B. Jacob, Clélia Margarete T. Borralho, Roberto Vito Ardito and [2]Milton Arthur Ruiz

[1]São Paulo State University - UNESP-IBILCE Street Columbus 2265, Sao José do Rio Preto, SP 15054-000 Brazil
[2]Institute of Cardiovascular Diseases - IMC - Rua Castelo D´Agua 3030 - São José do Rio Preto SP Brazil 15015-210

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ABSTRACT

Worldwide are held about 16,000 transplants of stem cells autologous and 29,000 allogeneic. In Brazil, the stem cell transplant autologous bone marrow transplantation increases about 1,000 per year. Cell therapy or transplantation of Hematopoietic Stem Cells (HSC) is a regenerative medicine that aims to restore and regenerate the functions of senescent cells or tissues with serious injuries. The CTH constitute from 0.05% to 0.1% of human bone marrow and circulating haematopoietic cells. The phenotype of hematopoietic stem cells include CD 34 antigen expression of the (+) and CD133 (+). Samples were collected in 120 ml (± 5.0 %) blood from bone marrow, from a universe sample consisted of fifteen patients and the comparison between the manual method and system SEPAX. For all samples the quantification of total leukocytes (CD34 (-)) ranged from 3.5 x 10^3 to 6.5 x10^3, no evidence of infection. The average measurements of cells extracted by SEPAX were statistically superior to the manual method and the technique SEPAX obtained a lower standard deviation of measurements, showing greater accuracy of the technique. Furthermore, there was no statistically significant correlation (p-value > 0.05) among these techniques, proving that the method can be SEPAX greater control of the results. Added to this, the technique SEPAX, achieved a higher viability of hematopoietic stem cells. Therefore, the system opens SEPAX advantages over manual by several factors, as well as ensuring the sterility of the product also allows extracting higher number of cells CD34 (+), with greater viability.

INTRODUCTION

Cell therapy and stem cell transplantation is the regenerative medicine that aims to restore and regenerate the functions of senescent cells or tissues with serious injuries (Asahara and Isner, 2002; Asahara *et al*., 1999; Asahara *et al*., 1997; Baldomero *et al*., 2010; Bhattacharya *et al*., 2000; Boitano *et al*., 2010; Boyer *et al*., 2000; Carmeliet and Luttun, 2001; Copelan, 2006; Davidoff *et al*., 2001). In Brazil, a large-scale clinical trial with 1,200 patients with heart failure received autologous mononuclear bone marrow, in 2005, funded by the Ministry of Health has confirmed the feasibility of cell therapy (Edelberg *et al*., 2002; Encontro, 2010; Ford *et al*., 2003; Fuchs *et al*., 2001; GIEBEL *et al*., 2010; Gill *et al*., 2001; Gunsilius, 2002; Hamano *et al*., 2001; Humeau *et al*., 1996; Ikenaga *et al*., 2001). The main pathologies that are subject to this treatment are heart disease, diabetes, cancer, lung diseases and genetic disorders (Ikpeazu *et al*., 2000; Kalka *et al*., 2000; Kerbauy, 2010; Lin *et al*., 2000; Marques *et al*., 2000; Mimeault *et al*., 2007). The use of cells derived from bone marrow angiogenesis experiments were performed in animal models of acute and chronic myocardial ischemia , using intracoronary via transendocardial and transepicardic (Orlic *et al*., 2001; Orlic *et al*., 2001; Perin *et al*., 2003; Ruiz, 2005). Animal models with critical ischemia of the lower limbs also had successful neovascularization (Ikenaga *et al*., 2001). Hematopoietic Stem Cells (HSCs) and Multipotent

Corresponding author: Idiberto José Zotarelli Filho,
State University of São Paulo - IBILCE-UNESP, Rua Cristovão Colombo 2265, São José do Rio Preto SP, Brazil 15054-000

Mesenchymal Cells (MSC) form the stem cell niche in the bone marrow that are important for the maintenance of stem cell pool and hematopoiesis (Asahara and Isner, 2002; Asahara *et al*., 1999; Asahara *et al*., 1997; Baldomero *et al*., 2010). The HSCs give rise to all the cells of the hematopoietic and immune systems and are characterized by expression of the surface molecule CD34 (+) and absence of markers of lymphoid and myeloid lineage (Bhattacharya *et al*., 2000; Boitano *et al*., 2010; Boyer *et al*., 2000; Carmeliet and Luttun, 2001; Copelan, 2006; Davidoff *et al*., 2001; Edelberg *et al*., 2002; Encontro, 2010; Ford *et al*., 2003). The HSC is defined as a cell with a high capacity for self- renewal and proliferative potential. Constitutes 0.05% to 0.1% of human bone marrow cells and hematopoietic cell current (Ford *et al*., 2003; Marques *et al*., 2000). The phenotype of hematopoietic stem cells include CD34 antigen expression of the (+) and CD90 (Thy -1) and the absence of CD38 (Ford *et al*., 2003; Marques *et al*., 2000). The antigen CD34 functions as an adhesion molecule Thy- 1 and is related to signal transduction gene. The HSC exhibits significant plasticity, and can convert from one type to another cell and non-hematopoietic cells differentiate (Fuchs *et al*., 2001; GIEBEL *et al*., 2010; Gill *et al*., 2001; Gunsilius *et al*., 2002; Hamano *et al*., 2001).

There is a subset of primitive HSC corresponding to the precursors of CD34 (+) cells and not expressed or expressed CD 34 trace amounts (Ford *et al*., 2003). These cells marker CD133 (+) and are the predominant part of a pool of quiescent hematopoietic and mesenchymal precursor cells. These CD34 (-) cells can differentiate into CD34 (+) cells circulate in the peripheral blood returned to the bone marrow, increasing progenitor cell population (Marques *et al*., 2000). The transplanted stem cells survive long keeping properties and are used for neoplastic diseases such as leukemias and lymphomas and also for non-neoplastic diseases such as aplastic anemia and autoimmune diseases (Ikpeazu *et al*., 2000; Kalka *et al*., 2000; Kerbauy *et al*., 2010; Schwella *et al*., 1995; Shi *et al*., 1998; Takakura *et al*., 2000). Parallel to this, you also need to perform the procedures for obtaining these cells in a closed system with high separation efficiency of hematopoietic stem cells and mesenchymal stem cells (mononuclear layer) (Lin *et al*., 2000; Orlic *et al*., 2001; Orlic *et al*., 2001; Perin *et al*., 2003).

Experimental Design

To make the samples of 120 mL (± 5.0 %) bone marrow blood, we started with a universe sample of fifteen patients. Samples were collected at the surgical hospital of cardiovascular diseases - HMC, after approval of the CEP. The processing of this material was carried out in the laboratory of cell therapy BMI/HMC and consisted in using the system SEPAX protocol (Ficoll-Paque) and the manual method with Ficoll-Paque. The results were analyzed with descriptive statistics and also statistics nonparametric Spearman correlation, using the software Minitab 15.1 and Program R.

MATERIALS

The materials used were try pan blue (Sigma Aldrich, St. Louis, MO, USA) tetrazolium salt (Sigma Aldrich, St. Louis, MO, USA) for MTT assay, Kit monoclonal antibody CD34 (+), CD34 (-), CD 133 (+) and CD 133(-) (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

Bone Marrow Harvesting

The patient was placed in lateral recumbency. The area of the pelvis was exposed, and fetal position was selected, that is, the lower limbs were bent under the trunk. Vital signs were checked every 5 minutes and oximetry was observed. All procedures were performed in conditions where there resuscitation of the patient. Was administered midazolam and anticipated sedative action. The hematologist was attired in a sterile manner and asepsis and antisepsis as recommendations for surgical procedures. Local anesthesia was administered also with 10 mL of 2 % lidocaine without vasoconstrictor, by distributing 5.0 mL each iliac crest, so as to anesthetize the periosteum an area to be punctured and not a single point. Alternatively, each iliac crest was anesthetized. Then it was placed 250 mL of saline in two different tanks, and one will be added in 2.0 mL of heparin. We prepared three 20 mL sterile syringes, previously washed in saline with heparin. The patient was then covered second surgical techniques. The clamp the collection bag was closed and added to 3.0 ml of heparin. Was collected at each aspiration at most 5.0 mL to improve the efficiency of collection. The liquid aspirated marrow was placed inside the bag with heparin.

After each puncture, the needle was washed Osgood. The bone marrow liquid was collected until the final volume to 125 mL. The clamps are opened and the bag by gravity, the bone marrow was filtered on line 850, 500 and 200 microns and packaged in a sterile pouch. By means of an output silicone were aliquoted 3.0 mL of the marrow aspirate for testing for sterility, cell viability and total cell count, CD 133 (+) and CD 34 (+). This was sealed, separate kit and packaged in a sterile field which was placed in the cooler with a thermometer to monitor the temperature of the box with a bone marrow sample from the operating room to the laboratory for cell therapy. On receiving the cell therapy laboratory, were confirmed by data sheets. The box was placed in the pass-through. The researcher vested in the laboratory led the box to the cleanroom where open purse and bone marrow was taken to a laminar flow hood (Shi *et al*., 1998; Takakura *et al*., 2000).

Processing Bone Marrow by Sepax

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 (+) and CD 133 (+), after obtaining the pellet by centrifugation (Aktas *et al*., 2008; Lapierre *et al*., 2007; Rodríguez *et al*., 2004).

Processing Bone Marrow by Manual

For the manual procedure separation layer mononuclear bone marrow, we used two conical centrifuge tubes of 50 ml each, and to each tube were added 15 mL of Ficoll-Hypaque and then slowly was added 30 ml of marrow bone. The assembly was led to the centrifuge 800 g for 10 minutes at room temperature (22 ^{0}C). After that, the mononuclear layer was aspirated with a Pasteur pipette with 1.0 cm and 1.0 cm plasma fraction of Ficoll-Hypaque and centrifuged again under the same conditions. Thereafter, the mononuclear cell layer was aspirated and on it were added replica with n = 3, 30 ml of a 5% physiological solution of albumin to remove Ficoll mononuclear cell layer. The final product were separated in 1.0 mL of test for quantifying CD 34 (+) and CD 133 (+), as well as cell viability assays (BOYUM, 1968).

Nucleated Cell Count - Leukocytes Total (CD 34(-))

The rate to be measured was diluted with Turk's solution at a ratio of 1: 40 and the procedure was done in replicates (n = 3), each counting was repeated if the difference between them exceed 10 %. The mixture was allowed to stand for 10 minutes. Were removed 10 L of this solution and placed in a Neubauer chamber for 3 minutes. This was then taken under an optical microscope for carrying out the counts in all four quadrants. The counting was performed using an electronic counter Bioplus in the program 02 (STEWART, 1996).

Cell Viability by Trypan

Aliquots quantified were diluted in trypan blue solution at a ratio of 1: 40 and the procedure was done in triplicate, with each score was repeated if the difference between them exceed 10 %. The mixture was allowed to stand for 10 minutes. Were removed 10 L of this solution and placed in a Neubauer chamber for 3 minutes. This was then taken under an optical microscope for carrying out the counts in all four quadrants. The counting was performed using an electronic counter Bioplus in the program 02 (Takakura *et al*., 2000).

Cell Viability by Mtt

Cell viability using the MTT assay was to assess the activity of the mitochondrial succinyl dehydrogenase as the enzyme present in the living cell capable of promoting the reduction of bromide 3 - (4,5-dimethylthiazol-2-yl) -2,5 - diphenyltetrazolium. Cell viability was assessed by reducing the bromide 3 - (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT) (Cat. M2128, Sigma) using resuspended in mononuclear cell layer polystyrene plates of 96 wells. Cells (5.0 x 10^{4}) was incubated at (37 ± 1%) ° C in humid atmosphere containing 5% CO_2. After 24 hours of incubation, the supernatant was discarded and added to each well MTT diluted in Eagle's minimum medium (Minimum Eagle Medium - MEM), and incubated the cells (37% ± 1) °C for 4h. Shortly thereafter, aliquots were trnasferidas 96-well plate in which the absorbance was read at 595 nm using a microplate reader ASYS EXPERT PLUS n = 10 replicates. Data were analyzed using the program PRISM (version 4:00), being used ANOVA followed by post-test and Dunnett's multiple comparison Neuman_Keuls with a significance level of 95% (Liu *et al*., 1997).

Quantification CD 34 (+) and CD 133 (+) / CD 34 (+/-)

The evaluation of the final purity and characterization of the cellular composition of hematopoietic stem cells from the bone marrow with markers CD 34 (+) and CD 133 (+) were performed by flow cytometry. About 2.0 x10^{5} cells immunomagnetically selected or total mononuclear cells (CD34 (+) or CD133 (+) cells) were resuspended in 200 µl of PBS and divided into two aliquots of 100 µl and incubated separately for two pipes, one control and one test. Test tube were added to 3µl of anti-CA 133 PE-conjugated fluorochrome followed by addition of 3µl of anti-CD34 antibody conjugated to FITC (fluorescein isothiocyanate) and the control tube was added 3 µl of solution nonspecific control antibody conjugated γ1 FITC (IgG1 isotype) and γ2 conjugated to PE (isotype IgG2A). About from 10,000 to 50,000 events were acquired and plotted as a function of the parameters FSC (forward scatter), which corresponds to the size of the cell and SSC (side scatter), which corresponds to the granularity. According to these parameters, the events corresponding to cells with typical profile lymphocyte progenitor cells were selected constituting the gate R1, while events do not correspond to selected artifacts or debris. The cells were then selected on R1 plotted on a dot plot where the signal labeled cells with γ1 and γ2 were used for instrument calibration. Finally, cells labeled with CD34-FITC antibody and AC133-PE were read on a new dot plot for the quantification of the fluorescence signal (STEWART, 1996; BOYUM, 1968).

Statistical Results

The statistical tools used in this study with a sample size of 30 samples of cells from the bone marrow mononuclear layer, subdivided into 15 samples obtained by the method SEPAX and 15 samples obtained by the manual method

(Altman, 1991). Descriptive statistics and normality test also Anderson-Darling (AD) for each subgroup in order to establish whether the measures are parametric and what the critical level of significance between the measurements. Then became Spearman correlation between the methods SEPAX and Manual, in order to know the correlation coefficient between the methods, as well as the level of significance between them (Armitage and Berry, 1994).

RESULTS

Quantification CD 34+ / kg and Quantification of total Leukocytes CD 34 (-)

For all samples the quantification of total leukocytes (CD34 (-)) ranged from 3.5 x 10^3 to 6.5 x10^3, no evidence of infection in any of the fifteen samples. Descriptive statistical analysis revealed by Figure 1 that the quantification of hematopoietic stem cells CD34 (+) per kilogram of patient was higher by SEPAX method, since the average value of the measurements by immunophenotyping was 2.91 x 10^6/ kg the SEPAX, with a confidence interval of 95 % from 2.7 to 3.3 x 10^6 (Figure 1) and 0.972 x 10^6/ kg for the manual, with a confidence interval of 95 % from 0.942 to 1.32 x 10^6 (Figure 1).

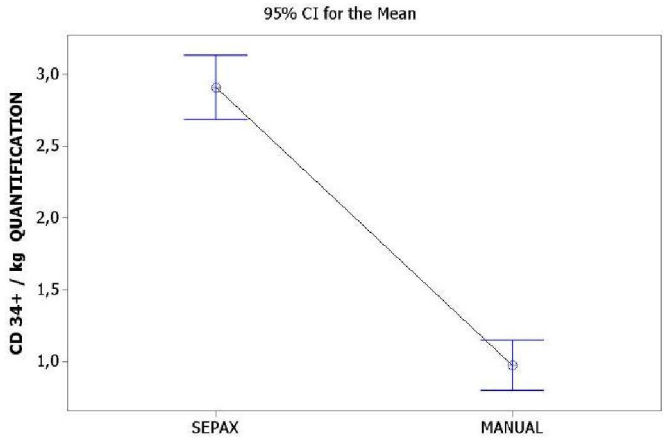

Figure 1. Graph representing the confidence interval and mean quantifying CD34 (+) / kg between methods SEPAX, with a confidence interval of 95 % from 2.7 to 3.3 x 10^6 and Manual, with a confidence interval of 95 % from 0.942 to 1.32 x 10^6

After the test of normality of each variable separately SEPAX and Manual, with a confidence interval of 99 %, coughing and perianal both measurements SEPAX as the Manual measurements did not show a normal distribution, confirmed by test Anderson-Darling (AD), Figure 2. SEPAX the system, measurements showed a standard deviation of 2.91 ± 0.404, AD = 0.900 and p-value = 0.016 < 0.05. Manual method, measurements showed a standard deviation of 0.97 ± 0.32, AD = 1.43 and p-value < 0.01 = 0.005. So, as measures of Anderson-Darling (AD) are greater than the values of p-value, it is evident that both the values of the system SEPAX as the manual method does not follow a Gaussian distribution, directing the studies of non-parametric Spearman correlation. Some authors advocate measurement values of CD34 (+) / kg patient from 0.5 x 10^6 to 2.5 therapy to be effective [13, 26]. Most authors show that cell therapy with hematopoietic progenitor cells from bone marrow obtained significant results with values quantifying CD34 (+) / kg up to 2.0 x 10^6 (Ford *et al.*, 2003; Marques *et al.*, 2000; STEWART, 1996).

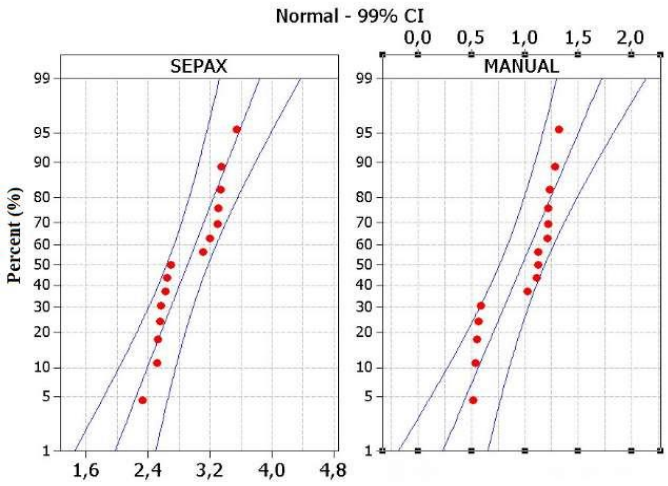

Figure 2. Graph showing the test of the normality of the measures to quantify CD34 (+) / kg by the methods SEPAX and Manual. SEPAX the system, measurements showed a standard deviation of 2.91 ± 0.404, AD = 0.900 and p-value = 0.016 < 0.05. Manual method, measurements showed a standard deviation of 0.97 ± 0.32, AD = 1.43 and p-value < 0.01 = 0.005

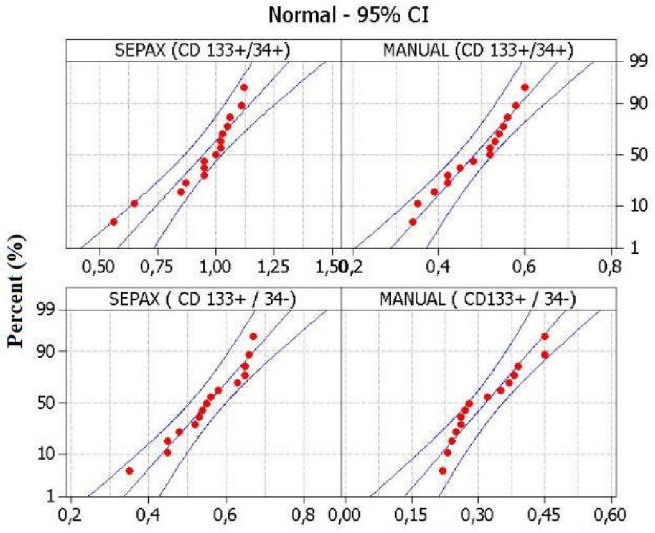

Figure 3. Graph showing the normality test measures quantifying CD 133 (+) / CD34 (+) and CD 133 (+) / CD34 (-) by the methods SEPAX and Manual. For SEPAX (CD 133 (+) / CD34 (+)), AD equal to 0.905 and p-value equal to 0.015. For Manual method (CD 133 (+) / CD 34(+)), AD equal to 0.402, p-value equal to 0.314. For SEPAX (CD133 (+) / CD34 (-), AD equal to 0.316 and p-value equal to 0.507. For Manual method (CD 133 (+) / CD 34 (-)), AD equal to 0.580 and p-value > 0.05= 0.110 The measures do not follow a Gaussian distribution, since the AD values are greater than p-value

Quantification CD 133(+) / CD 34(+ / -) / kg

For quantification CD 133 (+) / CD34 (+) / kg by SEPAX method, the average of the measurement was 0.946 x 10^6, the standard deviation equal to 0.1589, AD equal to 0.905

and p-value equal to 0.015. Already by the method of quantifying the average manual CD 133 (+) / CD34 (+) / kg was equal to 0.4833, standard deviation equal to 0.0835, AD equal to 0.402, p-value equal to 0.314. Quantification CD 133 (+) / 34 (-) / kg by SEPAX obtained by the method of quantification average equal to 0.5513, standard deviation equal to 0.0927, AD equal to 0.316 and p-value equal to 0.507, and the manual method had an average quantification equal to 0.3147, standard deviation equal to 0.0784, AD equal to 0.580 and p-value > 0.05= 0.110. So, as measures of Anderson-Darling (AD) are greater than the values of p-value, it is evident that both the values of the system SEPAX as the manual method does not follow a Gaussian distribution, directing the studies of non-parametric Spearman correlation (Figure 3).

Correlation Methods Sepax and Manual

All measures reported above non-normal distribution, it was non-parametric Spearman correlation between the measures and the methods SEPAX and Manual (Figures 4 and 5). The Spearman correlation coefficient between the measures SEPAX and Manual for quantifying CD34 (+) / kg was r = -0.2952 and the critical level of significance was p > 0:05 = 0.2854.

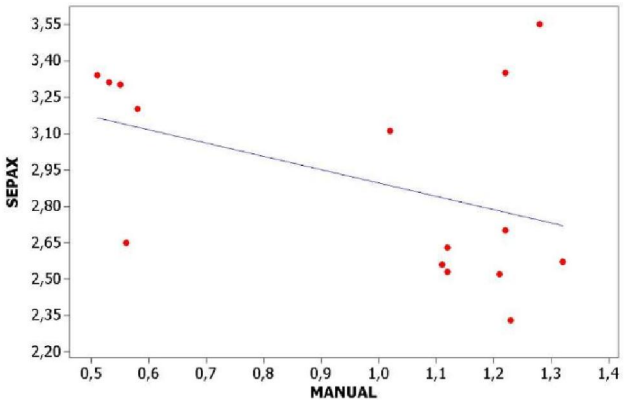

Figure 4. Graph showing correlation nonparametric Spearman between the two variables, SEPAX and manual. The correlation coefficient between the measurements was r = -0.2952 and the critical level of significance was p > 0.05 = 0.2854

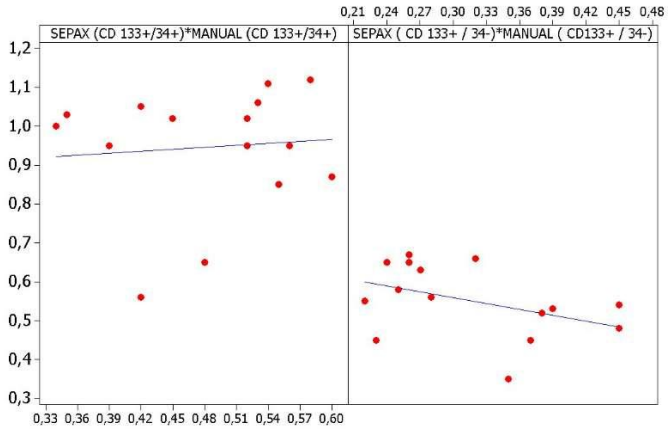

Figure 5. Graph showing correlation nonparametric Spearman between the two variables, SEPAX and manual. Spearman coefficient = 0.0620, p-value = 0.8263 for SEPAX manual / CD133 (+) / 34 (+); Spearman coefficient = -0.3880, p-value = 0.1529 for SEPAX / Manual CD 133 (+) / 34 (+/-)

The Spearman correlation coefficient to quantify CD 133 (+) / CD34 (+) / kg was equal to r = 0.0620, p-value > 0.05 = 0.8263, among the methods SEPAX and Manual. Already the Spearman coefficient for quantification CD 133 (+) / CD34 (-) / kg was equal to r = 0.3880, p-value > 0.05 = 0.1529 between methods SEPAX and Manual.

Cell Viability by Mtt and Trypan

For studies of cell viability with MTT, the average value for the measures of SEPAX was equal to 94.2 %, standard deviation equal to 3.0 %, AD equal to 0.535, p-value of 0.142. As for the manual method, the average value was equal to 87.6 %, standard deviation equal to 3.7 %, AD equal to 0.484 and p-value equal to 0.194. For viability studies with trypan blue, the average cell viability with SEPAX was equal to 92.93 %, standard deviation equal to 3.49 %, AD equal to 0.264 and p-value equal to 0.647. For the Manual, the average value was equal to 86.4 %, standard deviation equal to 4.8 %, equal to 0.521 AD and p-value equal to 0.155. The Spearman correlation coefficient for cell viability assays between MTT-Sepax and MTT-Manual was equal to = -0.0766 and p-value > 0.05 = 0.7862, and among Trypan- SEPAX and Trypan-Manual was equal to r = -0.2149 and p-value > 0.05 = 0.4418 (Figure 6).

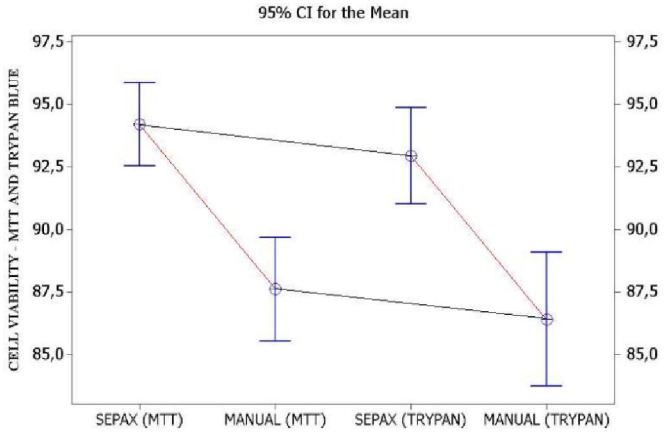

Figure 6. Graph showing the confidence interval and average measures of cell viability of cells in layer mononuclear using trypan blue and salt tetrazolium (MTT) methods SEPAX and Manual

DISCUSSION

The extraction of hematopoietic stem cells from bone marrow SEPAX method was greater due to the accuracy of the technique, in addition to obtaining a greater number of cells, the cells also ensures greater cell viability. Added to this, the system processes the SEPAX cellular material in a closed environment, providing greater security and inhibiting contamination. The normality test, using the statistical tool of Anderson-Darling (AD) and values of p-value for each group of samples and SEPAX Manual, showed a profile nonparametric, which led to the use of the correlation coefficient Spearman between groups SEPAX and manual measurements relative to CD34 (+) and CD 133 (+) / CD 34 (+ / -). The Spearman correlation coefficient (r) for all comparisons of measurements SEPAX / Manual, showed up near zero and the critical level of significance (p-value) of all the correlations presented with p > 0.05. This shows that there is low correlation between the techniques

and SEPAX Manual, revealing that the quantification of stem cells taken from the hematopiéticas technical SEPAX are larger and there are relations of similarities between the results. The values of both MTT cell viability with Trypan also confirmed as the biggest security method SEPAX forward the Manual, as well as to obtain average cell viability above 90 % with SEPAX, this technique also showed no significant correlations ($p > 0.05$) with the technical manual and getting results far better than the manual technique.

Conclusion

From the presented results and discussion, it is concluded that the extraction of hematopoietic stem cells by the method SEPAX showed no statistically significant correlations with cell extracts from the same manual method. Thus, the quantification of cell CD34 (+) and CD 133 (+) / CD 34 (+ / -) technique SEPAX values were far higher than the same technique for the quantification Manual, SEPAX system pointing as favoring processing of hematopoietic stem cells.

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REFERENCES

Asahara T, Isner JM. Endothelial progenitor cells for vascular regeneration. *J Hematother Stem Cell Res*. 2002; 11:171-8.

Asahara T, Masuda H, Takahashi T, *et al*. Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization. *Circ Res*. 1999; 85:221

Asahara T, Murohara T, Sullivan A, *et al*. Isolation of putative progenitor endothelial cells for angiogenesis. *Science*. 1997; 275:964-7.

Baldomero, Helen. *et al*. Hematopoietic Stem Cell Transplantation: A Global Perspective, *The Journal of the Americam Medical Association, Chicago*, v. 303, n. 16, p. 1617-1624, mai. 2010.

Bhattacharya V, Mc Sweeney PA, Shi Q, *et al*. Enhanced endothelialization and micro vessel formation in polyester grafts seeded with CD34 (+) bone marrow cells. *Blood*. 2000; 95:581-5.

Boitano, AE, Wang J, Romeo R, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science* 2010; 329:1345-1348.

Boyer M, Townsend LE, Vogel LM, *et al*. Isolation of endothelial cells and their progenitor cells from human peripheral blood. *J Vasc Surg*. 2000; 31:181-9.

Carmeliet P, Luttun A. The emerging role of the bone marrow-derived stem cells in (therapeutic) angiogenesis. *Thromb Haemost*. 2001; 86:289-97.

Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med*. 2006; 354:1813-26.

Ministério da Saúde (BR). Portaria GM nº 2600 de 21 de outubro de 2009. Aprovao Regulamento Técnico do Sistema Nacional de Transplantes. Brasília, DF; 2009.

Davidoff AM, Ng CY, Brown P, et al. Bone marrow-derived cells contribute to tumor neovasculature and, when modified to express an angiogenesis inhibitor, can restrict tumor growth in mice. *Clin Cancer Res*. 2001; 7: 2870-9.

Edelberg JM, Tang L, Hattori K, Lyden D, Rafii S. Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. *Circ Res*. 2002; 90:E89-93.

Encontro de Diretrizes Brasileiras em Transplante de Células Tronco Hematopoéticas, I. Sociedade Brasileira de Transplante de Medula Óssea. Rev Bras Hematol Hemoter. 2010; 32: (supl.1).

Ford CD, Chan KJ, Reilly WF, Petersen FB. An evaluation of predictive factors for CD34+ cell harvest yields with chemotherapy and growth factors. *Transfusion* 2003; 43:622-625.

Fuchs S, Baffour R, Zhou YF, *et al*. Transendocardial delivery of autologous bone marrow enhances collateral perfusion and regional function in pigs with chronic experimental myocardial ischemia. *J Am Coll Cardiol*. 2001; 37:1726-32.

GIEBEL, Sebastian; *et al*. Association of Human Development Index with rates and outcomes of hematopoietic stem cell transplantation for patients with acute leukemia. *Blood Journal, Washington*, v.116, n. 1, p. 122-128, abr. 2010.

Gill M, Dias S, Hattori K, *et al*. Vascular trauma induces rapid but transient mobilization of VEGFR2(+) AC133(+) endothelial precursor cells. *Circ Res*. 2001; 88:167-74.

Gunsilius E. Bone marrow-derived endothelial cells for therapeutic angiogenesis and antiangiogenesis: facts and visions. *J Hematother Stem Cell Res*. 2002; 11:153-5.

Hamano K, Li TS, Kobayashi T, *et al*. The induction of angiogenesis by the implantation of autologous bone marrow cells: a novel and simple therapeutic method. *Surgery*. 2001; 130:44-54.

Humeau L, Bardin F, Maroc C, Alario T, Galindo R, Mannoni P, Chabannon C. Phenotypic molecular, and functional characterization of human peripheral blood CD 34 +/Thy1+ cells. Blood 1996; 87:949-955.

Ikenaga S, Hamano K, Nishida M, et al. Autologous bone marrow implantation induced angiogenesis and improved deteriorated exercise capacity in a rat ischemic hindlimb model. *J Surg Res*. 2001; 96:277-83.

Ikpeazu C, Davidson MK, Halteman D, Browning PJ, Brandt SJ. Donor origin of circulating endothelial progenitors after allogeneic bone marrow transplantation. *Biol Blood Marrow Transplant*. 2000; 6:301-8.

Kalka C, Asahara T, Krone W, Isner JM. Angiogenesis and vasculogenesis. Therapeutic strategies for stimulation of postnatal neovascularization. Herz. 2000; 25:611-22.

Kerbauy, Fábio Rodrigues; RIBEIRO, Andreza Alice F. Transplante de células tronco hematopoiéticas. In: HAMERSCHLAK, Nelson (Org.). Manual de hematologia. Barueri: Manole, 2010. cap. 38, p. 299- 304.

Lin Y, Weisdorf DJ, Solovey A, Hebbel RP. Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest*. 2000; 105:71-7.

Marques JV, Vigorito AC, Aranha FJ *et al*. Early total white blood cell recovery is a predictor of low number of apheresis and good CD34+ cell yield. *Transfus Sci* 2000; 23(2):91-100.

Mimeault M, Hauke R, Batra SK. Stem cells: a revolution in therapeutics-recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies. *Clin Pharmacol Ther*. 2007; 82(3):252-64. Revisão.

Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci*. 2001; 938: 221-9; discussion 229-30.

Orlic D, Kajstura J, Chimenti S, *et al*. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc Natl Acad Sci* U S A. 2001; 98:10344-9.

Perin EC, Dohmann HF, Borojevic R, *et al*. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. Circulation. 2003; 107:2294-302.

Ruiz MA. A era da terapia celular. *Rev. Bras. Hematol. Hemoter*. 2005; 27(1):4.

Schwella N, Siegert W, Buyer KJ *et al*. Autografting with blood progenitor cells: predictive value of pre-apheresis blood cell counts on progenitor cell harvest and correlation of the reinfused cell dose with hematopoietic reconstitution. *Ann Hematol* 1995; 71:227-234.

Shi Q, Rafii S, Wu MH, *et al*. Evidence for circulating bone marrow-derived endothelial cells. Blood. 1998; 92:362-7.

Takakura N, Watanabe T, Suenobu S, *et al*. A role for hematopoietic stem cells in promoting angiogenesis. Cell. 2000; 102:199-209.

Liu Y, Peterson DA, Kimura H, Schubert D. Mechanism of cellular 3-(4,5-dimethylthiazol 2-yl)- MTT and in vitro anti-rabies evaluation 17 2,5-diphenyltetrazolium bromide (MTT) reduction. *J Neurochem*, 1997, 69(2): 581-93.

STEWART, C. C. & STEWART, S. J. The use of directly and indirectly labeled monoclonal antibodies in flow cytometry. In: Methods in Molecular Biology, vol 45: Monoclonal antibody protocols, ch. 15. 1996. Totowa, NJ: W.C. Davis Humana Press Inc.

BOYUM, A. Separation of leukocytes from blood and bone marrow. *Scand. J. Clin. Lab. Invest*., 1968, 97, 7.

Aktas M, Radke TF, Strauer BE, Wernet P, Kogler G. Separation of adult bone marrow mononuclear cells using the automated closed separation system Sepax. *Cytotherapy*. 2008; 10(2): 203-11.

Lapierre V, Pellegrini N, Bardey I, Malugani C, Saas P, Garnache F *et al*. Maddens S. Cord blood volume reduction using an automated system (Sepax) vs. a semi-automated system (Optipress II) and a manual method (hydroxyethyl starch sedimentation) for routine cord blood banking: a comparative study. *Cytotherapy*. 2007; 9(2):165-9.

Rodríguez L, Azqueta C, Azzalin S, García J, Querol S. Washing of cord blood grafts after thawing: high cell recovery using an automated and closed system. Vox Sang, 2004; 87(3): 165-72.

Altman, D. G. Practical Statistics for Medical Research, Chapman & Hall, 1991, London.

Armitage, P. and Berry, G. Statistical Methods in Medical Research, 1994, 3rd ed., Blackwell, Oxford.

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