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Original Article

Impact of donor characteristics on the quality of bone marrow as a source of mesenchymal stromal cells

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Abstract: In recent years, the therapeutic use of mesenchymal stromal cells (MSC) has generated a valuable number of scientific studies that delve into their biological characteristics and their potential in regenerative medicine; however, the impact of the clinical characteristics of tissue donors, from which these cells are isolated, on their potential in applied clinical research is not yet clear. The objective of this study was to evaluate the impact of the clinical characteristics of bone marrow donors on the quality of this tissue as a source of MSC for therapeutic use. Human MSC were isolated, characterized and cultured (according to ISCT criteria) from bone marrow samples from volunteer donors (n = 70) attending the Department of Orthopedics and Traumatology of the Hospital Universitario San Ignacio (Bogota, Colombia) for surgery of prosthetic hip replacement that agreed to participate voluntarily in the study. Donor data such as age, gender, weight, smoker and type of anesthesia used during the surgical procedure were recorded, and the impact of these characteristics on the volume of tissue collection, mononuclear cell count and confluence time of cells with fibroblastoid morphology was evaluated. Correlation coefficients between quantitative variables were calculated with Spearman's correlation test, and the association between qualitative and quantitative variables was evaluated with biserial correlation coefficient. A significant correlation was observed between the age of the donors and the time necessary to obtain confluent cells in vitro ($r = 0.2489$, $P = 0.0377$); similarly, the correlation between the volume of bone marrow collected and the number of mononuclear cells obtained was significant ($r = 0.7101$, $P = 0.0001$). Although a negative correlation tendency was observed between the mononuclear cell count and the confluence time, this was not significant ($r = -0.2041$, $P = 0.0950$). No significant associations were observed between gender, smoking status or type of anesthesia and the expansion characteristics of human mesenchymal stromal cells. Bone marrow donor age and the tissue collection volume impact the time of obtaining MSC in vitro and the mononuclear cell count with which the culture starts. These conditions must be considered when the bone marrow is selected as the tissue for obtaining MSC.

Keywords: Mesenchymal stromal cells, human bone marrow, quality criteria

Introduction

For several years, mesenchymal stromal cells (MSC) have been considered a therapeutic option in regenerative medicine. Several authors have discussed the lack of standardized procedures for the in vitro expansion of MSCs and the effect this has on their proliferation capacity, phenotype, secretome, expression of adhesion molecules and anti-inflammatory effect [1-4]. In addition to diversity in the expansion protocols of these cells, other factors such as the type of source tissue and the clinical characteristics of the donors are crucial to guarantee an optimal collection and culture of MSC. Although, in recent years, multiple tissues have been described as potential sources of MSC, human bone marrow (hBM) remains one of the most studied and most used tissues to obtain this cell population. Previously, reports have shown that there are differences in the morphology, glucose consumption and lactate and ammonium production between MSCs isolated from different healthy BM donors [5] and that the age and gender of BM donors influence the in vitro function of MSCs derived from this tis-

Table 1. Characteristics of voluntary donors of human bone marrow (n = 70)

Variable	Range (%)	Mean ± Standard Deviation	Median
Age (years)		56.52 ± 12.77	59
Weight (Kg)		63.52 ± 18.21	61
Gender			
Female	49 (70)		
Male	21 (30)		
Smoker			
Yes	7 (10)		
No	63 (90)		
Anesthesia			
General	57 (81)		
Spinal	13 (19)		
Bone marrow volume (ml)		35.67 ± 19.37	35

sue [6]. Additionally, in our group the differences between MSC of bone marrow of different anatomical places were demonstrated [7]. The objective of this work was to evaluate the impact of bone marrow donor characteristics such as age, gender, weight, smoking status and type of anesthesia used during the surgical procedure on the quality of bone marrow as a source to obtain MSCs.

Materials and methods

Clinical characteristics of bone marrow donors

Clinical data of volunteer donors (age, gender, smoking status and type of anesthesia) and human bone marrow (hBM) samples were obtained with the support of the Department of Orthopedics and Traumatology of Hospital Universitario San Ignacio (Bogota, Colombia). The bone marrow donors (n = 70) attended for hip replacement surgery and agreed to participate voluntarily in the study after signing the informed consent form approved by the Hospital Ethics Committee. After exposure of the femoral head bone or acetabular subchondral bone, an average volume of 35 ml of bone marrow was collected (Mean 35.67 ± 19.37) in a sterile tube containing ethylene diaminetetraacetic acid anticoagulant (EDTA). The samples were processed immediately to obtain hBM-MSC.

Isolation, characterization and culture conditions for hBM-MSC

hBM mononuclear cells were isolated by ficoll density gradient centrifugation (Histopaque d = 1.077 g/cm^3, Sigma-Aldrich, USA) and then counted in the Micros 60 automated hematology analyzer (Horiba ABX). Cells were plated at a density of 1.6 × 10^5 cells/cm^2 in IMDM Glutamax-I (GIBCO, Invitrogen) according to previously published protocols [8-10]. hBM-MSC phenotype was assessed by flow cytometry in a FACS Aria-II cytometer (BD Biosciences) with the following monoclonal antibodies (MAbs): FITC mouse anti-human CD73 (clone AD2, BD Pharmingen™), PE mouse anti-human CD105 (clone SN6, Invitrogen), PerCP mouse anti-human CD45 (clone 2D1, BD Biosciences) and APC mouse anti-human CD34 (clone 581, BD Pharmingen™). For data analysis, DIVA (BD Biosciences) and FlowJo software were used. hBM-MSC functional assay was performed according to previous reports [9, 10] with Stem Pro Osteogenesis, Stem Pro Adipogenesis and Stem Pro Chondrogenesis Differentiation Kits (Invitrogen). Confluence time of the cell culture (first pass) was determined as the time necessary to obtain adherent cells (fibroblastoid morphology) with a confluence greater than 70% (cells obtained from the mononuclear cells initially cultured).

Statistical analysis

Statistical analysis was performed by the GraphPad Prism version 5.0 (GraphPad) and XLSTAT. The Shapiro-Wilk test was used to assess the normality of the data. Spearman's correlation test was calculated to ascertain the relationship between donor age and volume of bone marrow collected, total mononuclear cells isolated and cell culture confluence time. Biserial rank association coefficient was calculated to determine the relationship between donor gender, smoking status and type of anesthesia and the expansion characteristics of human mesenchymal stromal cells. A *p*-value of less than 0.05 was considered significant for all analyses.

Results and discussion

Bone marrow samples were collected from 70 volunteer donors. The characteristics of the individuals are presented in **Table 1**. After obtaining samples, hBM-MSCs were isolated, cultured and characterized. All cultures per-

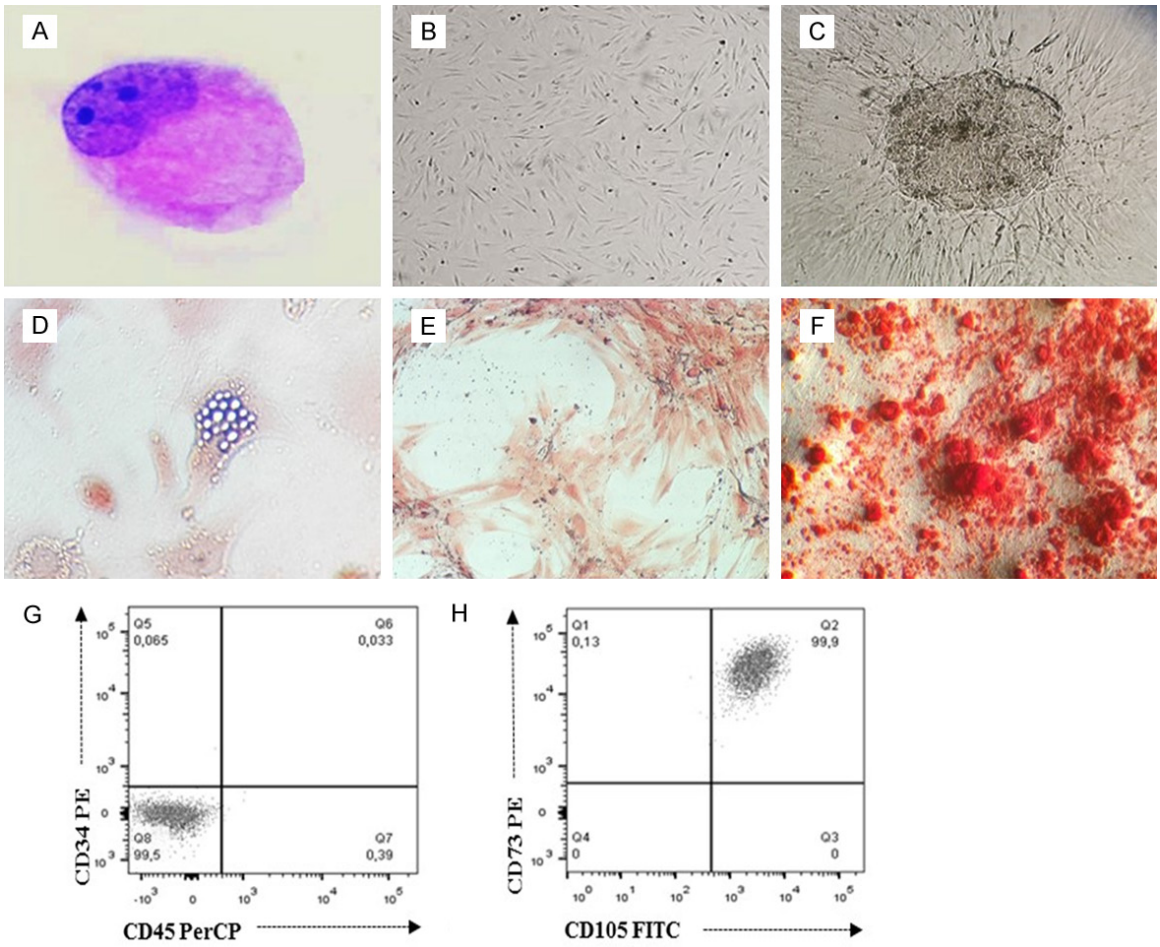

Figure 1. MSC characteristics isolated from human BM. (A) MSC morphology (3 passage): cells with an eccentric nucleus, loose chromatin and nucleoli; presence of granular basophilic cytoplasm (× 100 Cythospin, hematoxylin and eosin stain, optical microscopy), (B) MSC morphology (3 passage): Fibroblastoid cells associated with MSC (× 20, inverted microscopy) and (C) fibroblast colony (× 20, inverted microscopy), (D) Adipogenic differentiation (× 20, sudan black stain, inverted microscopy), (E) Chondrogenic differentiation (× 20, safranin O stain, inverted microscopy), (F) Osteogenic differentiation (× 20, red alizarin stain, inverted microscopy), (G, H) Immunophenotype (3 passage): MSCs do not express hematopoietic antigens (CD34 and CD45) and co-express CD73 and CD105.

Table 2. General characteristics of mesenchymal stromal cell cultures isolated from human bone marrow (n = 70)

Variable	Range (%)	Mean ± Standard Deviation	Median
Total mononuclear cells isolated (× 10^6/ml)		34.29 ± 27.59	26
Cell culture viability (%)		99.83 ± 0.48	100
Cell culture confluence time (days)		13.6 ± 3.74	13
Fibroblastoid colonies (%)			
Yes	23 (33)		
No	47 (67)		
Immunophenotype (according to ISCT) (CD34-/CD45-/CD73+/CD105)			
Yes	70 (100)		
No	0		

formed met the criteria established by ISCT in 2006 [11]: fibroblastoid morphology, immunophenotype (CD34-/CD45-/CD73+/CD105+) and osteogenic, chondrogenic and adipogenic

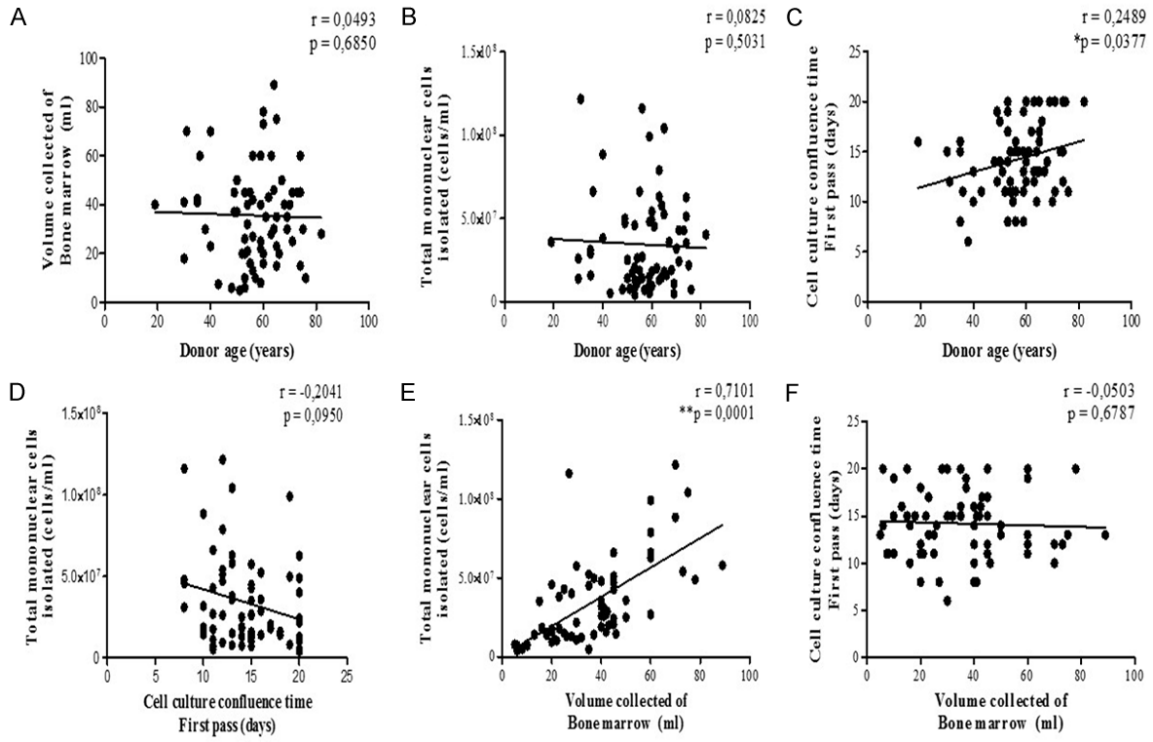

Figure 2. Correlation between clinical characteristics of bone marrow donors and isolated mesenchymal stromal cells. (A-C) Donor age (years) vs. BM volume, total mononuclear cells isolated and cell culture confluence time, (D) Total mononuclear cells isolated vs. cell culture confluence time, (E, F) BM volume vs. total mononuclear cells isolated and cell culture confluence time.

Table 3. Coefficients of biserial correlation

Donor characteristics	Biserial correlation parameters	Volume collected of bone marrow (ml)	Total mononuclear cells isolated (× 10^6/ml)	Cell culture confluence time (days)
Gender	r	-0.075	0.130	-0.126
	Valor-p (bilateral)	0.545	0.294	0.306
Smoker	r	-0.114	-0.166	-0.153
	Valor-p (bilateral)	0.356	0.151	0.189
Anesthesia	r	0.084	0.147	-0.112
	Valor-p (bilateral)	0.511	0.216	0.347

Donor characteristics and mesenchymal stromal cells in vitro conditions (alpha value: 0.05).

differentiation capacity (**Figure 1**). **Table 2** shows the results of the cultivated hBM-MSC characteristics.

A significant correlation was observed between the age of the donors and the time necessary to obtain confluent cells in vitro (r = 0.2489, P = 0.0377); similarly, the correlation between the volume of bone marrow collected and the number of mononuclear cells obtained was significant (r = 0.7101, P = 0.0001). Although a negative correlation tendency was observed between the mononuclear cell count and the confluence time, it was not significant (r = -0.2041, P = 0.0950) (**Figure 2**). Non-significant correlation was observed between the age of the donors and the volume of bone marrow collected or the total isolated mononuclear cells; similarly, non-significant correlation was observed between the BM volume and the confluence time (**Figure 2**). Additionally, non-significant associations were observed between gender, smoking status and type of anesthesia and the expansion characteristics of human mesenchymal stromal cells (**Table 3**).

Additionally, non-significant associations were observed between gender, smoking status and type of anesthesia and the expansion characteristics of human mesenchymal stromal cells (**Table 3**).

Currently, there are many promising studies on the clinical applications of hBM-MSC [2, 12-20]; however, there are also a high number of discrepancies and failed results in clinical trials with these cells [21-23]. Now, it is clear that the source tissue, in vitro expansion strategies, the state of differentiation, dosage and the existence of different cellular subpopulations in the group of "mesenchymal cells" (stromal cells vs. stem cells) can influence the experimental results with these cells [24]; however, it is not yet clear how the characteristics of the tissue donors from which MSCs are isolated can influence their potential for expansion and/or therapeutic capacity. Alt et al. previously demonstrated that the age of the donor negatively influences the multipotent capacity of mesenchymal stromal cells isolated from adipose tissue [25]. Similarly, Du et al. demonstrated that the proliferative and osteogenic capacity of periodontal ligament stromal cells decreases with the age of the donor [26], and Sun et al. have shown that aged MSCs produce extracellular matrix proteins that contribute to their own aging [27]. In our study, we found that the age of the donor influences the time necessary to obtain BM adherent cells similar to fibroblasts. This parameter is important since a slow expansion of mesenchymal stromal cells in vitro can also indicate cell aging events that may affect the regenerative potential of these cells [28]. Another important parameter for the efficient expansion of hBM-MSC is the volume of bone marrow; we found that the higher the volume collected is, the greater the number of mononuclear cells to start the cultures. We consider that to efficiently isolate hBM-MSC, it is necessary to use tissue volumes between 30 ml and 35 ml (**Figure 2**). Previously, it has been shown that the volume of tissue influences the efficiency of isolation of mesenchymal stromal cells, and this has been demonstrated with umbilical cord blood [29, 30]. We did not find an association between other characteristics of the donors such as gender, smoker or type of anesthesia and the variables of in vitro expansion of mesenchymal stromal cells. Our study contributes to the knowledge of the pre-analytical conditions that must be considered when using bone marrow as a source tissue for the isolation of mesenchymal stromal cells.

Conclusions

Our work contributes to the knowledge about the pre-analytical conditions necessary for the in vitro expansion of MSC obtained from BM. Clinical characteristics of bone marrow donors, such as age, and sample conditions, such as the volume of cells collected, are important factors for the establishment of protocols related to the efficiency of the expansion of MSC in vitro.

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Disclosure of conflict of interest

None.

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