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Core diameter of bone marrow aspiration devices influences cell density of bone marrow aspirate in patients with severe peripheral artery disease

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Abstract

Background aims. Bone marrow (BM) transplantations are an accepted therapeutic strategy for hematologic conditions. In the past decades, interest for BM-derived cell therapy has extended toward the field of regenerative medicine. Irrespective of the treatment strategy, its success depends on the amount of cells available for transplantation. Both patient and procedural factors have been shown to influence the cell density of the BM aspirate. In the present study, the influence of core diameter of the BM aspiration device on cell density of the BM aspirate is studied. *Methods.* BM harvesting procedures performed in a clinical trial investigating the effect of BM cell therapy in patients with severe peripheral artery disease were retrospectively studied (clinicaltrials.gov NCT00371371). Patients underwent BM harvesting through the use of either a 15-gauge ($n = 85$) or an 8-gauge ($n = 75$) needle. The numbers of harvested white blood cells (WBC) and $CD34^+$ hematopoietic cells (HPC) were quantified. *Results.* The amount of WBC per milliliter of BM aspirate was significantly higher when the 8-gauge needle (27.8×10^6 WBC/mL [95%CI $25.4–30.5 \times 10^6$]) was used compared with the smaller 15-gauge core needle (20.1×10^6 WBC/mL [95% confidence interval (CI), $18.7–21.7 \times 10^6$], $P < 0.001$). For the amount of $CD34^+$ HPC, a similar pattern was observed (185×10^3 HPC/mL [95% CI, $161–213 \times 10^3$]; 114×10^3 HPC/mL [95% CI, $96–134 \times 10^3$]; $P < 0.001$). *Conclusions.* The application of a BM aspiration device with a larger core diameter is associated with an increased cell density of the BM aspiration product in patients with severe peripheral artery disease.

Key Words: *bone marrow, bone marrow aspiration, peripheral artery disease, stem cells*

Introduction

Bone marrow (BM) aspirations have been performed for several decades to obtain stem and progenitor cells for BM transplantations (BMTs) to treat malignant and nonmalignant hematologic disorders. More recently, stem and progenitor cell therapy has gained interest as potential regenerative medicine (RM) strategy for cardiovascular and orthopedic diseases. Successful allogeneic hematopoietic BMT depends on the total number of nucleated donor cells transplanted [1,2]. The number of administered BM cells has also been suggested to be an important determinant of clinical effectiveness in RM therapies [3–6].

Because higher BM aspiration volumes and more puncture sites are related to increased risk for complications [7,8], strategies to optimize cell density of the harvested BM are of large clinical relevance. Patient related factors, such as body weight (BW) and peripheral white blood cell (WBC) count, have been reported to influence the cell density of the obtained BM [9]. However, these patient characteristics cannot easily be influenced, in contrast to procedural factors. Limiting the aspiration volume

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per site or the use of an aspiration needle with additional side-holes [10–12] are procedural factors that have been associated with an increased density of the BM product.

In a retrospective study of 160 BM harvests for the Juventas Trial (clinicaltrials.gov NCT00371371) [13,14], a randomized, double-blind, placebo-controlled trial that investigates the efficacy of repeated intra-arterial infusion of BM mononuclear cells (BM-MNC) in patients with no-option severe limb ischemia, we evaluated the influence of patient characteristics and the core diameter of the BM aspiration needle on the cell density of the harvested BM product (also see Supplementary material for the Juventas Trial).

Methods

Study population

Data on 160 BM harvestings of patients participating in the Juventas Study between September 2006 and July 2012 were analyzed. The Juventas Study is a clinical trial evaluating the clinical effects of intra-arterial infusion of BM-MNC in no-option severe limb ischemia (clinicaltrials.gov NCT00371371) [13,14]. Patients with chronic severe limb ischemia, an ankle-brachial index of 0.6 or less, or an unreliable index (non-compressible or not in proportion to the Fontaine classification) and who were not candidates for conventional revascularization were included in this trial. Exclusion criteria were a history of neoplasm or malignancy in the past 10 years, concomitant disease with life expectancy of less than 1 year, inability to obtain sufficient BM aspirate, known infection with human immunodeficiency virus, hepatitis B or C virus and an impossibility to complete follow-up.

The study was approved by the local Internal Review Board of the University Medical Center Utrecht, and written informed consent was obtained from all patients.

BM aspiration procedure

Patients were administered fentanyl and midazolam to induce conscious sedation. Xylocaine 2% was used for additional local anesthesia at the BM aspiration site. BM aspiration was performed by an experienced hematologist at the right iliac crest, which was identified by manual palpation. The BM needle was inserted into the BM compartment, and 8 to 10 mL of BM was collected; after advancing the needle somewhat deeper into the compartment again, 8 to 10 mL of BM was obtained. Thereafter, the needle was relocated to another part of the iliac crest and the identical procedure was repeated until approximately 100 mL of BM was obtained. The BM was collected in bottles containing 50 mL of saline with 100 IU/mL sodium heparin. Subsequently, BM-MNCs were isolated by use of density gradient separation (DGS) with the use of Ficoll-Paque (GE Healthcare) in the Cell Therapy Facility of the University Medical Center Utrecht, according to Good Manufacturing Practice–graded protocols.

From September 2006 to February 2011, a 15-gauge BM aspiration needle (Lettix B.V.) was used unless the length of the device was insufficient to reach the BM compartment. From March 2011 routine practice shifted toward the use of a larger 8-gauge needle (Angiotech Pharmaceuticals Inc).

Cell quantification and characterization

The number of WBCs per milliliter was counted with the use of an automatic cell counter (Coulter Ac*T 8, Beckman Coulter), and the total number of WBCs was calculated by multiplying by the total volume of collected BM.

$CD34^{+}$ hematopoietic progenitor cells (HPCs) were quantified by means of flow cytometry (FACS Calibur, BD Biosciences). A BM volume containing 1×10^{6} WBCs was incubated with monoclonal anti-human $CD45^{PerCP}$, $CD34^{PE}$, $CD14^{FITC}$ and $CD66^{FITC}$ antibodies. Erythrocytes were lysed with standard lysis solution. Cells in the lymphocytic range that were CD34/CD45 double-positive and negative for both CD14 and CD66 were characterized as HPCs. Additionally, we quantified the number of a putative endothelial progenitor cell (EPCs staining CD34/KDR double-positive [15]) in an identical fashion. The number of HPCs was expressed as percentage of the total number of WBCs counted. To calculate the total number of HPCs, the percentage obtained by means of flow cytometry was multiplied by the total number of WBCs assessed with the use of the automatic cell counter.

Statistical analyses

Normality of continuous variables was explored through the use of histograms and Q-Q plots. Equality of variances was tested by use of Levene's test for variances. In the case of non-normality, data were log-transformed and again tested for their normality characteristics and equality of variances. Data are expressed as means and 95% confidence intervals (95% CI) for normally distributed data or medians and interquartile ranges (IQR) for data that retained a non-normal distribution even after transformation. Log-transformed data were reconverted to a linear scale to express their geometric means and