HEMATOL TRANSFUS CELL THER. 2018;xxx(xx):xxx–xxx

HEMATOLOGY, TRANSFUSION AND CELL THERAPY

www.rbhh.org

Original article

# The influence of cell concentration at cryopreservation on neutrophil engraftment after autologous peripheral blood stem cell transplantation

*Olga Margareth Wanderley de Oliveira Félix*[a], *Gisela Tunes*[b], *Valéria Cortez Ginani*[c], *Paulo César Simões*[a], *Daniele Porto Barros*[a], *Elizabete Delbuono*[a], *Maria Teresa de Seixas Alves*[d], *Antonio Sérgio Petrilli*[a], *Maria Lúcia de Martino Lee*[a], *Roseane Vasconcelos Gouveia*[c], *Victor Gottardello Zecchin*[a], *Adriana Seber*[c,*]

[a] *Instituto de Oncologia Pediátrica, Grupo de Apoio ao Adolescente e a Criança com Câncer (GRAACC), São Paulo, SP, Brazil*
[b] *Universidade de São Paulo (USP), São Paulo, SP, Brazil*
[c] *Hospital Samaritano de São Paulo, São Paulo, SP, Brazil*
[d] *Universidade Federal de São Paulo (Unifesp), São Paulo, SP, Brazil*

ARTICLE INFO

*Article history:*
Received 13 April 2016
Accepted 22 January 2018
Available online xxx

*Keywords:*
Cryopreservation
Stem cell transplantation, peripheral
Autografts
Pediatrics
Dimethyl sulfoxide

ABSTRACT

*Background:* Peripheral blood stem cell concentrations are traditionally adjusted to 20–40 × $10^6$ leukocytes/mL prior to freezing. This low cell concentration at cryopreservation implies larger volumes with more dimethyl sulfoxide being used, and higher cost and toxicity at the time of transplant. Higher cell concentrations have been reported but this is not widely accepted. Moreover, the influence of cell concentration on engraftment has not been well documented. Therefore, this study retrospectively analyzed the influence of peripheral blood stem cell concentration at freezing on engraftment after autologous hematopoietic stem cell transplantation.

*Method:* Leukapheresis products were plasma-depleted and cryopreserved with 5% dimethyl sulfoxide, 6% hydroxyethylamide solution and 4% albumin in a −80 °C freezer. Individual patient data from hospital records were reviewed.

*Results:* Fifty consecutive patients with oncological diseases underwent 88 leukaphereses. Median age was six years (range: 1–32 years) and median weight was 19 kg (range: 8–94 kg). Median leukocyte concentration was 109 × $10^6$/mL at collection and 359 × $10^6$ (range: 58–676 × $10^6$) at freezing with 78% viability (range: 53–95%); leukocyte recovery after thawing was 95% (range: 70–100%). In multivariate analysis, cell concentration ($p$-value = 0.001) had a negative impact on engraftment. Patients infused with bags frozen with <200 × $10^6$ leukocytes/mL engrafted after a median of nine days (range: 8–12 days), 200–400 × $10^6$ leukocytes/mL after 11 days (range: 9–20 days); 400–600 × $10^6$ leukocytes/mL after 12 days (range: 8–19 days) and with cell concentrations >600 × $10^6$ leukocytes/mL, engraftment was after 14 days (range: 13–22 days).

* *Corresponding author at*: Rua Conselheiro Brotero, 1486, São Paulo, SP, CEP: 01232-010, Brazil.
E-mail address: seberadriana@gmail.com (A. Seber).

https://doi.org/10.1016/j.htct.2018.01.007



Please cite this article in press as: Félix OM, et al. The influence of cell concentration at cryopreservation on neutrophil engraftment after autologous peripheral blood stem cell transplantation. Hematol Transfus Cell Ther. 2018. https://doi.org/10.1016/j.htct.2018.01.007

*Conclusion:* In patients with adequate CD34 cell collections, total leukocyte concentrations of $282 \times 10^6$/mL, freezing with 5% dimethyl sulfoxide and 6% hydroxyethylamide solution without a controlled-rate freezer, and storing cells at −80 °C yielded excellent engraftment. Further increases in cell concentration may delay engraftment, without affecting safety.



## Introduction

Cryopreservation of hematopoietic stem cells (HSC) has been used for many years to maintain their viability and proliferative potential for autologous hematopoietic stem cell transplants (HSCT). Bone marrow (BM) or peripheral blood stem cell (PBSC) concentration is usually adjusted before freezing in a cell suspension with 20–40 × $10^6$ leukocytes/mL.[1]

Dimethyl sulfoxide (DMSO) is an intra-cellular cryoprotectant that can maintain cell viability after thawing, but it is associated to many side effects.[2] Four methods have been used to decrease the toxicity of the DMSO infusion: divide the cell infusion over consecutive days, to wash out the DMSO prior to transplant, to decrease the DMSO concentration at freezing,[3] and finally, to concentrate the cells in order to have a smaller volume to be frozen and consequently, less DMSO to be infused.[4–8] The Fred Hutchinson Cancer Research Center published their experience freezing cells at higher concentrations (median 370 × $10^6$ leukocytes/mL) over 20 years ago, with good viability and engraftment.[1] Other centers have used two cryoprotectants, combining intracellular and extracellular activity [5% DMSO and 6% hydroxyethylamide solution (HES)], freezing cells without a controlled-rate freezer, and storing bags at −80 °C until the time of transplant.[6] For more than a decade, many children were transplanted at the Instituto de Oncologia Pediátrica-Grupo de Apoio ao Adolescente e a Criança com Câncer (GRAACC) of the Universidade Federal de São Paulo (Unifesp) using the latter method combined with high cell concentrations without a single graft failure, but the impact of using different cell concentrations on engraftment has not been adequately addressed.

## Objective

The objective of this paper is to retrospectively evaluate the impact of cell concentration at freezing on neutrophil engraftment.

## Method

This is a retrospective study of 50 consecutive patients with neoplastic diseases and complete laboratory data submitted to HSCT with autologous PBSC at the GRAACC. All parents or guardians signed the institutional consent form for leukapheresis and for the transplant. This study was approved by the Institutional Ethics Research Committee (CEP 047/08).

The leukapheresis products were completely plasma-depleted after centrifugation at 3000 rpm (2616 × *g*) for 15 minutes at room temperature. The leukocytes remaining in the bag were not adjusted for any specific predetermined concentration. Patients with a very high CD34 cell count in the peripheral blood, who were expected to undergo only one leukapheresis procedure, had at least 30 mL remaining in the bag, so PBSC were frozen in at least two canisters for safety reasons. The cells were cryopreserved in a solution of DMSO (Edwards Lifesciences Research Medical®, Irvine, CAN) and HES as previously described,[6] with a final concentration of 5% DMSO, 6% HES and 4% human albumin. The bags were placed into metal canisters, transferred to an −80 °C freezer and, up to 2002, stored in this freezer until used. After 2002, the bags were transferred to a nitrogen tank. As these products had already shown adequate viability, cell recovery and engraftment, a controlled-rate freezer was not installed in 2002 because it would increase costs.

Quality control before freezing included automated and differential leukocyte count (ACT Diff Beckman, Coulter Fullerton, CAN or micros 60 – CT Horiba® ABX, Montpellier, FRA), cell viability testing using 0.4% Trypan Blue dye exclusion (Gibco BRL, NY, USA) in a cell counter chamber (BOECO®, Neubauer, DEU), and CD34 cell counting, all performed before centrifugation. Sterility tests for aerobic bacteria and fungi used 3 mL of the final supernatant (pediatric bottles – Bactec® – Becton, Dickinson and Company, County Clare, IRL), which was cultured for seven and 30 days, respectively. CD34 positive cells were counted in a Facs Calibur® flow cytometer (Becton Dickinson, San Jose, CA, USA) using anti-CD45-FITC and anti-CD34-PE (BD, Biosciences, San Jose, CA, EUA) monoclonal antibodies, according to the ISHAGE protocol.[9] On the day of the transplant, cells were thawed in the laboratory in a 37 °C water bath (Precision Scientific – Winchester, VA, USA) in a sterile plastic wrap (Sterifarma Produtos Cirúrgicos Ltda, São Paulo, Brazil).

Two thawing methods were used according to the attending physician's request, removing the DMSO or not. All patients who would receive a DMSO dose greater than 1 g/kg or who were less than 20–25 kg in weight, and those who had renal, respiratory or cardiac insufficiency, had the DMSO removed from the bag prior to the infusion using the previously validated New York Blood Center procedure to remove DMSO from cord blood products.[10] The cells were rapidly infused though the central line without filters or infusion pumps. Patients were monitored (heart rate, respiratory rate, oxygen saturation) from the beginning of the infusions until two hours after the end of the last bag. All side effects were

Please cite this article in press as: Félix OM, et al. The influence of cell concentration at cryopreservation on neutrophil engraftment after autologous peripheral blood stem cell transplantation. Hematol Transfus Cell Ther. 2018. https://doi.org/10.1016/j.htct.2018.01.007