Effect of cryopreservation on CD34+/CD45- cell count in canine bone marrow from dogs with lymphoma that underwent autologous bone marrow transplantationjury

Juliana Santilli[a], Maria Luísa B. de Cápua[b], Aureo E. Santana[c], Ana Paula M. N. Canesin[d], Sabryna G. Calazans[a]

[a] Franca University (UNIFRAN), Franca, SP – Brazil
[b] Bioethicus Institute, Botucatu, SP – Brazil
[c] College of Agricultural and Veterinary Science (FCAV), São Paulo State University (UNESP), Jaboticabal, SP – Brazil
[d] Educational Center Barão de Mauá, Ribeirão Preto, SP – Brazil

Abstract

Autologous transplantation involves temporary removal of the hematopoietic stem cells (HSC) from the receptor itself. The HSC are derived from bone marrow or peripheral blood and can be reinfused in the receptor after induced myelosuppression by chemotherapy or radiation. However, cryopreservation of bone marrow or peripheral blood bags may reduce the number of nucleated cells. Therefore, it is important to quantify these cells after freezing, prior to infusion into the patient. The aim of this study was to quantify CD34+/CD45- stem cells by means of flow cytometry in dogs with lymphoma that underwent autologous bone marrow transplantation, before and after cryopreservation. Seven dogs diagnosed with lymphoma were included in this study. The dogs were 3-9 years old and weighed 12 to 39Kg. Three dogs were male and four dogs were females. There were two Rottweilers, two mongrel dogs, one Labrador Retriever, one Cocker Spaniel and one Golden Retriever. All patients were treated with the Madison-Wisconsin protocol. After induction phase, dogs that achieved complete remission had the bone marrow collected from iliac crest. The transplantation protocol consisted of bone marrow harvesting (10 mL/kg), followed by administration of cyclophosphamide (400 or 500 mg/m^2) and intravenous reinfusion of bone marrow. Bags containing bone marrow were processed in steps including red cell depletion, plasma depletion and cryoprotectors addition. After homogenizing the contents of the freezing bag, samples of 1ml were separated to quantify stem cells before the freezing process. Bags were stored at -80°C for four days. After the freezing process, the viability of the cells was evaluated. We used the "International Society of Hematotherapy and Graft Engineering" (ISHAGE) protocol for quantitation of CD34+ stem cells. Analyses were performed in duplicate with monoclonal antibody anti-CD45+ conjugate to isothiocyanate of fluorescein (FITC) (Rat Anti-Dog CD45: FITC, SEROTEC) and anti-CD34+ conjugated with phycoerythrin (PE) (PE Mouse Anti-Dog CD34, BD Pharmingen) analyzed with FACSDiva software FACSCANTO on the flow cytometer (Becton Dickinson, San Jose, CA, USA) for identification and quantification of CD34+CD45- cells. The t-test was used to detect difference between moments.

The mean values and standard deviations of HSC (CD34+/CD45-), before and after freezing were 0.145 (± 0.171) x 10^6/kg and 0.09 (±0.108) x 10^6/kg, respectively. The difference between these periods was not significant ($p > 0.05$). We did not find any description that showed the stem cells quantification in bone marrow bags of dogs in similar conditions to our methodology.

Cryopreservation did not influence the count of HSC (CD34+/CD45-) in bone marrow bags stored at -80°C for four days, reinfused in dogs with lymphoma.

Ethics Committee: FCAV/UNESP n° 17265-06.