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Evaluation of the manual process of DMSO removal in cryopreserved hematopoietic progenitor cells of pediatric patients in Hospital de Clinicas of Porto Alegre

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Introduction: The maintenance of cryopreserved cell viability depends on its resistance to damage caused by dehydration and by mechanical damage due to the formation of ice crystals inside the cells. One way to prevent this damage is by using cryoprotectants during the freezing process, with dimethyl sulfoxide (DMSO) being the most often used intracellular cryoprotectant in the cryopreservation of hematopoietic progenitor cells (HPC). The use of DMSO is often associated with toxic effects to the patient, such as nausea, vomiting, chills and an unpleasant sensation in the oropharynx. Dyspnea, cardiac dysfunction, anaphylaxis and acute renal failure are rarer effects. It is recommended that the dose of DMSO/kg of patient should not exceed 1g/kg/day. As the toxic effects of DMSO are more common in pediatric patients and the dose of DMSO/kg is associated with the toxicity degree, its removal is indicated for these patients. Objective: To evaluate the DMSO removal process in cryopreserved HPC of pediatric patients from April/2011 to May/2016. Material and Methods: Thirty-nine HPC of pediatric patients were cryopreserved using DMSO, hydroxyethyl starch (HES) and human albumin at final concentrations of 5%, 6% and 3% respectively. After defrosting the HPC at 37 ° C, the manual process of DMSO removal was performed, consisting in adding a wash solution at the proportion of 1: 1 at final concentrations of 2.25% of HES and 2.5% of human albumin, centrifuging at 400g for 20 minutes and removing the supernatant. Cell counts were performed in a hematologic counter. The analyzed parameters were: patients' age and weight, duration of DMSO removal procedure, volume and total nucleated cells (TNC) pre- (pre) and post-removal (post) of DMSO, cell recovery and dose of DMSO/kg. The Shapiro-Wilk's and the Spearman's correlation tests were used. Data are presented as median and interquartile range. Results: Age (years) and weight (kg) of patients were 4 (2-8) and 16.5 (13-24), respectively. Regarding HPC, the results were: time of processing (hours), 1:30 (1:15-2:01); volume pre (mL) 219.5 (161-407); volume post-removal (mL) 81.4 (61.2 to 143.7); TNC pre (x108) 400.0 (236.3 to 663.0); TNC post-removal (x108) 359.4 (201.8 to 548.5); cell recovery (%) 88 (80-94); dose of DMSO (g DMSO/kg patient) 0.82 (0.67 to 0.92). No correlation was found between the analyzed parameters. Discussion: The dose of pre-removal DMSO/Kg was high, although lower than the recommended maximum of 1g/kg/day, with the removal being indicated for pediatric patients. The obtained results demonstrate that manual removal of DMSO was efficient in relation to the TNC recovery and adequate regarding the time of processing. Conclusion: Manual removal of DMSO is a feasible procedure in laboratory practice and is recommended for pediatric patients due to the reduction of adverse effects during the infusion of cryopreserved HPC.

Keywords: cryopreservation, DMSO, pediatric transplantation