## POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER AFTER AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION IN A ADULT PATIENT: CASE REPORT

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**Introduction:** Post-Transplant Lymphoproliferative Disorder (PTLD) is a complication of allogeneic Bone Marrow Transplantation (BMT). Rare cases of PTLD after autologous BMT have been reported only in adults. This case report described PTLD in a patient after autologous Peripheral Stem Cell Transplantation (PSCT), and this condition is rare in patients who do not have HIV and are late after transplantation. **Materials:** We report a case of EBV lymphoproliferative disease 2-years after autologous transplantation for Diffuse Large B-Cell Lymphoma germinal center subtype. **Results:** This 66-year-old male with stage IVB Lymphoma Diffuse Large Cell B underwent autologous PSCT post first relapse. On day 650 post PSCT, patient started with night sweats, afternoon fever, asthenia and increased abdominal volume. On physical examination, no lympfondomegaly was found, but a voluminous splenomegaly was identified with 10 cm below the left costal margin. Opportunistic infections research was conducted: negative CMV PCR, Leishmaniose negative, Chagas negative, IGRA para Tuberculose negative, Toxoplasmose Negativo, HIV, HTLV, Hepatite B e C negative, HHV6 negative. PCR polymerase was conducted on whole blood EBV DNA, and DNA levels increased up to $2.11\times10^3$ copies/mL. Laboratory studies have shown normal levels of IgG, IgA, IgM. The bone marrow biopsy immunohistochemical analysis showed that the lymphocytes were positive for CD20, according to the immunophenotype below: Flow cytometric immunophenotyping analysis of the specimen revealed that the proportion of surface: 86.58% of the B lymphocytes (1.66% of the total cells analyzed) expressed CD19, CD20 (strong), CD23 (weak), CD45 (strong), CD79b, CD81 and lambda light chain restriction, without expression of CD10, CD38, CD43, CD200, CD305 or other markers investigated. 0.12% were plasma cells without immunophenotypic alterations (ckappa/clambda: 1.47). The patient presented a probable clinical presentation of EBV lymphoproliferative disease after autologous transplantation-mononucleosis like, the patient was treated with weekly rituximab and monitoring of EBV levels, with complete negative viral load and complete remission of the condition after 4 doses of rituximab. Monthly PCR monitoring for EBV with an undetectable viral load was performed after 6-months of evolution. **Conclusion:** New approaches for diagnosis and monitoring based on quantitative polymerase chain reaction for EBV DNA are being explored. What exactly is being measured (the source and character of the viral DNA) has yet to be determined, as well as the compartment that should be analyzed (whole blood, serum, plasma, or lymphocytes). Despite these issues, there is an emerging consensus that these technologies will facilitate rapid diagnosis and therapeutic monitoring in the future. A myriad of therapeutic interventions are or will be available. Rituximab, alone or in addition to other therapies, promises a profound change in the landscape with respect to the treatment and perhaps prevention of post-transplant lymphoproliferative disease.

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## UNRELATED PEDIATRIC UMBILICAL CORD BLOOD TRANSPLANTATION

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Unrelated umbilical and placental cord blood (UCB) is an effective source of hematopoietic stem cells, replacing bone marrow or peripheral blood stem cells when there is no related donor or matched unrelated adult available. To improve engraftment of these units with very low cellularity, the New York Cord Blood Bank (NYBC) has developed a method of thawing with slow dilution and washing of the cells based on *in vitro* data (Rubinstein, 1995), to reduce the osmotic damage caused by the density gradient at the time of infusion if cells are infused with no manipulation. Objective: This study is to report a two decade the experience using UCB as a source for hematopoietic reconstruction. Methods: Data was collected from the Cell Processing forms. The UCB units were received from national and international cord blood banks. The Dimethylsulfoxide (DMSO) removal was performed according to Rubinstein's protocol: after almost complete thawing in a water bath at 37°C, a cold (4°C) 5% dextran-albumin solution was slowly added to the bag in a 1:1 ratio on an icy surface, with continuous manual homogenization. An aliquot was removed for quality control and the cells were transferred to another bag and centrifuged at 400g 4°C for 20 min. The supernatant was transferred to a new bag and the buffy coat was resuspended in the same dextran-albumin solution and sent for infusion with a simple tubing. The supernatant was recentrifuged and infused until $< 10^7$ cells/kg remained. Results: Between Jun, 2000 to Sept, 2020 we received 47 UCB units, 34 of them from international banks (17 from the NYBC) and the others from two national banks. We performed 40 transplants in 37 patients with benign and malignant hematological diseases. Seven patients received a double UCB graft. The median age was 6-years (range: 1–18), weight 18 kg (7–68), and 25 patients were male. The median cellularity infused was after thawing and washing was $4.6\times10^7$/kg (1.7–17.2). The median time of engraftment was 10-days (range: 6–59) and 47% of the patients are alive. Six patients died without engraftment. An additional patient had his autologous UCB infused after a private bank collection and did not have hematopoietic reconstitution. Conclusion: The infusion of UCB is feasible when there are no other

donors available, however, the logistic of requesting, receiving, storing and manipulating the cells require an experienced team in the cell processing center and in the management of the patients.

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## EXPERIENCE OF A PEDIATRIC SERVICE IN THE STATE OF SÃO PAULO REQUESTING AND RECEIVING UNRELATED HEMATOPOIETIC STEM CELLS

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A major challenge for transplant centers is the graft acquisition when a compatible unrelated donor is chosen for the procedure. In addition to the complex logistics that involve several critical steps and the need of an experienced multidisciplinary team, another important challenge is the variability of hematopoietic stem cell collection methods that may or may not compromise neutrophil engraftment. Objective: This study is to describe the experience receiving unrelated Hematopoietic Stem Cell (HSC) products in a single pediatric center. Methods: This is a retrospective study of all unrelated HSC products received from national and international collection centers. Data were collected through specific forms from the Registry of Voluntary Bone Marrow Donors (REDOME) and data from the Cell Processing Center. Results: A total of 179 stem cell products have been received to date (December 2009 to April 2024) coming from national (n = 144) and international (n = 35) institutions, to treat leukemias (n = 129) and benign hematological diseases (n = 50). Most national collections were performed within our southeast region (65%) and most international collections came from Europe (n = 24), 68%, 18 of them from Germany, 8 of USA, 2 of Asian and 1 of South Africa. Only six grafts could not be infused fresh and were cryopreserved for later use. The bone marrow was the predominant stem cell source (n = 163). The unrelated donor activity progressively increased until 2019, the year with 21 transplants, but during the COVID-19 pandemic (2020 and 2021) dramatically decreased to 12 and 3 collections. The number of unrelated transplants is increasing again but with a high rate of refusal or significant delays of marrow collections on top of the national shortage of marrow collection bags and therefore, many Peripheral Blood (PBSC) collections have been accepted, although they are not our first choice. The unrelated donor transplants are 20% of our current transplant activity and 40% of the allogeneic ones. The cell number of 33/163 (20%) marrow collections were lower than the $5\times10^8$ leukocytes/kg requested, median $3.9\times10^8$/kg (1.2–4.8). Of the 14 PBSC products, the lowest cellularity requested was $5\times10^6$ CD34/kg and only two collections were lower than that, 2.3 (Brazilian) and $3.7\times10^6$ CD34/kg (international). Today, 64% of the patients remain alive. There were no reports of complications during transportation or failures in the process of identification of the recipient and donor numbers. Conclusion: In the past 15-years, with the 179 grafts received for unrelated donor transplants, we can describe a satisfactory graft most of the time, but worrisome significant delays and a shift from marrow to PBSC collections, that are known to have a higher chance of severe chronic graft versus host disease and negatively impact of the children's quality of life.

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## VALIDATION OF THE USE OF HYDROXYETHYL STARCH (VOLUVEN 6%) TO REMOVE DIMETHYL SULFOXIDE FROM HEMATOPOIETIC STEM CELLS

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During the process of cryopreservation of hematopoietic stem cells (CTH), the Dimelthyl Sulfoxide (DMSO) is responsible for ensuring a better cell viability and a satisfactory engraftment but, at the time of the graft infusion, it can cause serious adverse events, particularly in young children, in patients with renal dysfunction, cardiac insufficiency, hemodynamic instability, or when the dose of DMSO is equal to or greater than 1g/DMSO/Kg of body weight. An effective way to avoid serious reactions is to remove the DMSO using a washing solution composed by 40% dextran and 4% albumin. We have used this protocol, initially developed by the New York Blood Bank to remove DMSO and improve engraftment of unrelated umbilical cord blood units, since 2011. However, in October, 2023 we were informed with a short notice about the discontinuity of the production of Dextran40 (Laboratório de Insumos Farmacêuticos Ltda – LIFE). Objective: To describe the validation of the DMSO removal of hematopoietic stem cells in a Cellular Processing Laboratory using solution hydroxyethyl starch (HAES)-Voluven® 6% and albumin 4%, comparing the results of removal using Dextran 40 solution with 4% albumin. Methods: We used 22 bags from 7 patients: 16 from leukapheresis of deceased patients and 6 collected as Donor Leukocyte Infusions (DLI) but unused due to the development of Graft-Versus-Host Disease (GVHD). All families gave authorization for the cells to be discarded or used in research. Two similar bags were chosen from the same patient, stem cell source, volume and cellularity for parallel thawing in a 37°C water bath and then adding one of the solutions (Dextran vs. Voluven) in each bag at a 1:1 ratio. A sample was removed for nucleated cell count (CNT) and cell viability. The bags were