alterations remains a challenge and should take into consideration other laboratory and clinical data.

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EFFECTS OF DARATUMUMAB (DARA), CYCLOPHOSPHAMIDE (C), THALIDOMIDE (T) AND DEXAMETHASONE (D) COMBINATION ON LYMPHOCYTE POPULATIONS OF TRANSPLANT ELIGIBLE NEWLY DIAGNOSE MULTIPLE MYELOMA PATIENTS

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Background: The CTD combination have both an immunomodulatory and immunosuppressive activity on multiple-myeloma (MM) patients (pts) treatment. The advance of immunotherapy has been demonstrated by the development ofnew agents like anti CD38-antibody Dara, that are increasing the overall and progression-free survival of MM pts. Daraeffect on the immune system was already described, but few studies analyzed specifically lymphocytes population. Wehypothesized that Dara-CTD combination could impact on lymphocytes subsets during treatment.Aims: The primary endpoint was to quantify subpopulations of lymphocytes in pts with newly diagnosed multiplemyeloma (NDMM) transplant eligible (TE) pts, during Dara-CTD treatment phases (induction, consolidation andmaintenance). Secondary endpoint was to describe B cells subsets during the same phases **Methods:** Peripheral blood of 14 pts at four different time points was collected: at diagnose, after four cycles of Dara-CTD, after two consolidation cycles post-autologous stem cell transplantation (ASCT) and before maintenance therapy.Flow cytometry was used to detect lymphocyte surface molecules including CD3, CD4, CD5, CD8, CD16, CD19, CD20,CD38, CD45 and CD56 in the scatter plot. B cells were isolated and subpopulations (naïve B cells, non-class switchedmemory B cells, class switched memory B cells, IgD-CD27- memory B cells and plasmablasts) were detected by CD20,CD24, CD27, CD38, CD45 and IgD. Statistics was performed using the SPSS®v25.0 **Results:** The pts median age was 55 range (41-65) years old, and 57% were female. The median of T, B and NKlymphocytes subsets at diagnosis were 1153 x $10^3/\mu$L, 205x $10^3/\mu$L and 284 x $10^3/\mu$L cells, respectively. After four cyclesof Dara-CTD the median of T, B and NK cells dropped significantly to 889 x $10^3/\mu$L, 12 x $10^3/\mu$L and 11 x $10^3/\mu$L,respectively ($p<0.05$). The number of the cells after two consolidation cycles post-ASCT, showed T cells full recovery(1087 x $10^3/\mu$L) while B and NK cells had weakly reconstitution (15 x $10^3/\mu$L and 34 x $10^3/\mu$L, respectively). Beforemaintenance therapy, the median of T, B and NK cells were 1456 x $10^3/\mu$L, 24 x $10^3/\mu$L and 33 x $10^3/\mu$L, respectively.Regarding B cell population, the median of naïve B cell decreased after 4 induction cycles from 32 x $10^3/\mu$L to 1 x $10^3/\mu$L.Then, after two consolidation cycles post-ASCT the number of B cell increased to 14 x $10^3/\mu$L and to 18 x $10^3/\mu$L beforemaintenance **Summary/Conclusion:** Lymphopenia have been shown with different protocols using Dara as single agent or incombination. The present study confirmed that there is a decrease in the number of different lymphocytes populations (T,B and NK) after induction therapy with Dara-CTD. The T cells number recovery after two consolidation cycles post-ASCT,but B and NK cells remain low after the same period. There was a slowly but continuously recovering of B and NK cells,suggesting that Dara-CTD combination allows lymphocytes reconstitution. Analyzing the B cells subpopulations, thenaive B cell was the first to show a more significant recovery, although it was below the reference range (33 – 259). Inconclusion, this is the first study that report the lymphocyte profile during Dara-CTD treatment. This preliminary datasuggest that Dara-CTD induces general lymphopenia on (T, B and NK) populations after induction phase. It wasidentified that T cells recovery was complete after two consolidations cycles while the recovery of B and NK cells wasslowly but continuously.

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ELEVATED CIRCULATING ENDOTHELIAL CELLS AND SUCCESS IN ENDOTHELIAL COLONY-FORMING CELLS ISOLATION

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Introduction: Circulating endothelial cells (CEC) have been associated with vascular injury and are described as potential biomarkers for cardiovascular disease (1). Besides, current optimized methodologies enable the isolation of a well-characterized subtype of endothelial progenitor known as Endothelial colony-forming cells (ECFCs) (2,3). Although ECFC isolation methodologies are well described; some discrepancies remain in relation to their isolation efficiency. **Aims:** To evaluate the isolation efficacy ECFCs and CEC frequency in human peripheral blood. **Methods:** The Ethics Research Committee of the University of Campinas approved the experimental procedures and all donors signed the informed consent form. CEC enumeration was assessed by flow

cytometry in the peripheral blood of 18 donors. 100 μL of blood (with a leukocyte concentration between 5 and 10 $\times 10^3/\mu$L) was incubated with monoclonal anti-human antibodies (CD45 PerCp, CD133 APC, CD31 FITC, and CD146 PE). FACS lysing solution was used for red blood cell removal. 300,000 events or the total volume of the tube were acquired using a FACSCalibur® flow cytometer (4). To determine the number of cells/μL, the percentage of events was multiplied per number of leucocytes and divided per 100. Cell culture from venous blood was used to isolate ECFCs which were confirmed by their cobblestone morphology and immunophenotyping for endothelial markers (CD31, CD146, CD309, and CD144). **Results:** ECFC isolation was successful in 9 out of 18 donors (1 male, 8 females; mean age: 35.8 ±11.1 years), and not successful in the other half of donors (2 male, 7 females; mean age: 37.7 ±9.7 years). CEC frequency was significantly higher (p=0.004) in the group with successful ECFC isolation when compared to the group that did not yield ECFCs (2.9 ±1.2 CECs/μL against 1.2 ±0,8 CECs/μL). This suggests that the success in ECFC isolation might be related to the number of CECs, and therefore ECFC mobilization into circulation may also be linked to vascular injury. **Conclusion:** Increased number of CECs might be associated with the success of ECFCs isolation.

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IMPORTÂNCIA DO CONSERVANTE TRANSFIXNA AVALIAÇÃO DE AMOSTRAS DE LÍQUIDO CEFALORRAQUIDIANO: EXPERIÊNCIA DE UM SERVIÇO DE CITOMETRIA DE FLUXO DE UM HOSPITAL UNIVERSITÁRIO

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Objetivos: O envolvimento do sistema nervoso central (SNC) por neoplasias hematológicas é considerado uma complicação grave em pacientes com leucemias e linfomas. A análise do líquido cefalorraquidiano (LCR) por citometria de fluxo (CF) é bastante estabelecida e possui papel importante na definição prognóstica e avaliação de resposta ao tratamento dessas doenças. Porém, a degeneração celular rápida que ocorre neste material leva à necessidade de processamento imediato da amostra. Nesse sentido, o conservante celular Transfix®(Cytomark) tem sido amplamente utilizado, pois as amostras de LCR podem ser processadas em até 18-72h, sem perda celular. Assim, o objetivo do presente estudo foi avaliar as amostras de LCR antes e depois do estabelecimento do protocolo de coleta com Transfix®, em um Hospital Universitário. **Material e métodos:** Foram incluídas no estudo 142 amostras de LCR sem Transfix® e 142 com Transfix®. As amostras sem Transfix® colhidas entre 11/2017 e 09/2019 foram encaminhadas ao setor de CF em seringas contendo 1-10mL de LCR. A partir de 10/2019, tubos de EDTA 4mL contendo 100uL de Transfix® foram encaminhados às unidades do Hospital. As amostras com Transfix® obtidas entre 11/2019 e 04/2021 foram colhidas diretamente no tubo de EDTA/Transfix®contendo 1-4mL de LCR. Os LCR sem Transfix® foram processados imediatamente, enquanto aqueles colhidos com Transfix® foram acondicionados a 2 a 8°C até o momento do processamento (máximo 72h). Para a análise estatística, foram incluídos apenas os LCR com mais de 10 células analisadas. A quantidade de células analisadas representa o valor presente no laudo, obtido pelo software Infinicyt™após a remoção de restos e agregados celulares. **Resultados:** A quantidade de células analisadas foi avaliada em 135 amostras de LCR sem Transfix® e 141 com Transfix®. A mediana de células no grupo sem Transfix® foi de 1.335 células (31 - 998.336 células) e do grupo com Transfix® foi de 1.347 células (20 - 1.000.000 células). Não houve diferença estatística entre os grupos (p = 0,279). Seis amostras sem Transfix® não apresentaram células e outra apresentou apenas quatro células. Já no grupo com Transfix®, não havia células em apenas uma amostra. **Discussão:** Para prevenir a perda celular que ocorre logo após a coleta do LCR, a técnica da coleta desse material em tubos EDTA/Transfix® tem sido amplamente utilizada com objetivo de detecção de células hematológicas neoplásicas por CF. De acordo com os resultados obtidos no presente estudo, não houve diferença na quantidade de células analisadas na fase pré e pós Transfix®. Porém, na fase pré Transfix® as amostras foram processadas imediatamente após a coleta. Já na fase pós Transfix® foi possível processá-las em até 72h. Essa vantagem traz tranquilidade e organização para o serviço responsável pela coleta e envio ao laboratório, assim como para o setor de CF. Além disso, na fase pré Transfix® houve maior número de amostras com ausência de células, enquanto na fase pós Transfix® este fato aconteceu apenas em uma ocasião. **Conclusão:** A experiência com o uso do conservante Transfix® para avaliação de LCR por CF tem sido positiva, pois facilita a coleta, o transporte e o processamento dessas amostras.

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INCLUSÃO DO ANTICORPO TCRB1 NO PAINEL LINFOPROLIFERATIVO T - EXPERIENCIA COM RELATO DE DOIS CASOS

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Objetivos: O diagnóstico de doenças linfoproliferativas T normalmente são um desafio devido a dificuldade de diferenciar