protocolos de preparação e marcação da amostra, bem como a compensação do equipamento e monoclonais utilizados foram realizados de acordo com o protocolo Euroflow e a aquisição da amostra foi realizada em equipamento FACS CANTO II em 8 cores. A análise dos dados foi realizada em software Infinicyt. **Resultados e discussão:** O estudo imunofenotípico de ambas as amostras revelou de células de linhagem B CD19++ CD79a+ CD22+ com o seguinte fenótipo: expressão positiva de CD20, forte expressão de CD10, CD38, CD81, ausência de marcadores de imaturidade TDT, CD34 e ausência de expressão de cadeias leves Kappa/Lambda. A análise morfológica demonstrou células linfoides de médio tamanho, citoplasma basofílico, escasso, vacuolizado e cromatina heterogênea, levemente condensada com nucléolos evidentes. A conclusão do laudo de imunofenotipagem foi descritiva, sugerindo diagnóstico diferencial entre LLA-B e Linfoma de Burkitt através de dados moleculares. Observa-se que o paciente apresenta fenótipo sugestivo de células linfoides B imaturas devido a expressão forte de CD10, CD38 e ausência de expressão de cadeias leves (Kappa/Lambda), no entanto, essas características também são de Linfoma de Burkitt, devido a ausência de marcadores de imaturidade. Esses casos representam um fenômeno biológico distinto, sendo recomendada a analise molecular para análise do rearranjo isolado de MYC, pois a translocação de MYC pode ser adquirida em um estágio imaturo de diferenciação, manifestando assim, características fenotípicas e morfológicas de LLA-B e Linfoma de Burkitt simultaneamente. O paciente em questão não teve o exame MYC avaliado pois foi a óbito. **Conclusão:** No presente caso estudado, a análise imunofenotípica auxiliou no processo de investigação da doença, garantindo a rápida execução no processo devido ao uso de painéis padronizados. No entanto, as análises moleculares seriam fundamentais para a conclusão diagnóstica e definição da melhor estratégia de tratamento.

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DISTINCTION BETWEEN ACUTE MYELOID LEUKAEMIA WITH MYELODYSPLASIA-RELATED CHANGES AND MIXED-PHENOTYPE ACUTE LEUKAEMIA: A DIAGNOSTIC CHALLENGE

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The revised 4th edition of the World Health Organization (WHO) classification of tumours of haematopoietic and lymphoid tissues is based on clinical, morphological, immunophenotypic and genetic features. A case of acute myeloid leukaemia with myelodysplasia-related changes (AML-MRC) in which there was a mixed phenotype of blasts (B-cell/myeloid) has been previously described. We hereby also report an acute leukaemia case that had conflicting morphological and immunophenotypic findings, regarding the revised WHO classification. Patient was a 77 year-old female investigating anaemia for the past few months. Complete blood count (CBC) showed macrocytic anaemia (haemoglobin: 8.3 g/l; MCV: 102 fl) and thrombocytopenia (116 x 10^9/l), with a normal leukocyte count. Bone marrow assessment was performed when the patient CBC showed circulating blasts. Bone marrow flow cytometry immunophenotyping (FCI) revealed two blast cell populations: one population (about 26% of events) had an immature B-cell phenotype with expression of cytCD22, cytCD79a, CD19, CD22, CD34, CD45(dim), CD58, CD81 (dim), CD200, HLA-DR, nuTdT, and an aberrant CD13 and CD123 coexpression; while the other population (about 23% of events) had an immature myeloid phenotype with expression of CD4(dim), CD13, CD25, CD33, CD34, CD58, CD81, CD117, CD123, CD200 and HLA-DR. Neither population expressed cytCD3, cytMPO, CD2, CD3, CD7, CD10, CD11b, CD11c, CD18, CD20, CD36, CD56, CD64 or CD138. If classified isolated, the myeloid population would correspond to an acute myeloid leukaemia (AML) with minimal differentiation and the B-cell population, to a B-lymphoblastic leukaemia/lymphoma (B-ALL). In addition to the FCI data, bone marrow morphological evaluation revealed hypercellularity with severe multilineage dysplasia and two types of blasts with different sizes and morphology, blast cells accounted for 58% of cellularity. Cytogenetic studies showed a karyotype with 45,Xc, t(3;21)(q26; q22). FCI diagnosis considering the EGIL scoring system would be of a biphenotypic acute leukaemia, however to fit into the WHO classification of a mixed-phenotype acute leukaemia (MPAL), the myeloid population would need to express MPO or two monocytic markers, which was not what we observed. Nevertheless, bone marrow morphological findings were suggestive of AML-MRC. There are overlapping features between MPAL and genetically defined AML-MRC. A case series of MPAL identified complex karyotype as the most common genetic abnormality and the WHO classification specifies that AML with complex karyotypes should be classified as AML-MRC. It is recommended that karyotype findings should not be the sole deciding factor to distinguish MPAL from AML-MRC and other factors such as clinical history, morphological findings, immunophenotype and presence of somatic mutations associated with other neoplasms should be considered when leukemic blasts meet criteria for MPAL and the karyotype analysis has myelodysplasia-related abnormalities. Since we did not have access to karyotype results at diagnosis, a descriptive report of FCI and morphological findings was provided. In conclusion, FCI diagnosis of MPAL in the presence of myelodysplasia-related morphological

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