

(CD45++) CD8+, com expressão parcial de CD4 e de CD3 de superfície, expressão de CD3 citoplasmático (CADEIA EPSILON), de marcadores T (CD2 CD5 CD7) e de antígenos citotóxicos / NK (CD56+ CD16-/ CD94+ PERFORIN+ - PERFIL NK ESTÁGIO 4 - CD56++ COM CD16-/ E CD57 NEGATIVO), fenótipo de células T efectoras (CCR7 negativo CD27-/ CD28-/ + CD45RA+ homogêneo), sem expressão de TCRS (TCR ALFA/BETA, CBETA1 e GAMA/DELTA negativos). Antígenos negativos: CD25 CD57 CCR7 CD11C CD45RO CD1A CD117 CD30 HLADR TDT CD10 (estes 2 últimos foram positivos na Imunohistoquímica). Nesse momento apresentava lesões de pele em face e tronco, com necrose de extremidades (asa do nariz), teve piora do quadro clínico e foi submetido à intubação orotraqueal. Amostra de líquido também infiltrada por células T/NK. Os achados fenotípicos associados ao quadro clínico levaram à conclusão diagnóstica de Linfoma de Células T/NK. Paciente não apresentou melhora clínica, evoluiu com Mucormicose e foi a óbito. **Discussão:** As doenças linfoproliferativas crônicas de linfócitos T e de células NK representam menos de 10% das doenças linfoproliferativas. Além da sua raridade, os maiores obstáculos à sua identificação e caracterização têm sido a diversidade e complexidade fenotípica destas células. Os linfomas de células T/NK extranodais são mais comuns em homens adultos (média de idade 44-54 anos), originam-se de células NK (ou em alguns casos de linfócitos T citotóxicos), e causam extenso dano vascular com necrose proeminente. Têm forte associação com infecção por Vírus Epstein-Baer (EBV), e envolvem trato aerodigestivo superior (principalmente cavidade nasal), pele e linfonodos. É um linfoma altamente agressivo, com baixas taxas de sobrevida e resposta pobre ao tratamento. **Conclusão:** A citometria de fluxo foi indispensável na elucidação diagnóstica, na separação das subpopulações T e NK normais e patológicas da amostra, e na rápida definição de linfoma com curso clínico grave e agressivo.

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IMMUNOPHENOTYPING PANEL REPOSITIONING: NEW WAYS TO ANALYZE ACUTE MYELOID LEUKEMIA BIOMARKERS AND THEIR RELEVANCE

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One of the greatest obstacles in the treatment of Acute Myeloid Leukemia (AML) is relapse, occurring in 40-50% of

younger patients and most elderly patients. The Leukemia Stem Cells (LSCs) model suggests that only a small population of cancer cells is responsible for treatment resistance and recurrence. This phenotype is characterized by cells capable of persevering through treatment and reconstituting the different neoplastic populations. Over the years many have suggested that only CD34+CD38- leukemia cells could be LSCs, however, there has been controversy in the field and some have suggested that CD34+CD38+ could also present these characteristics. In a more recent time frame, CD123 is another marker that has been consistently associated with LSCs in AML. Currently, the immunophenotyping panels available for diagnosis and MRD (recently updated as measurable residual disease) detection focus solely on the detection or not of blasts (abnormal/neoplastic cells in the bone marrow). These strategies do not take into account valuable information regarding the biomarkers expression profile of each patient. In this context, we propose a new set of AML blast cells analyses format by repositioning the already world-widely used Euroflow panel for AML diagnosis and MRD detection. We performed a retrospective study with 262 patients diagnosed with AML (primarily) in the Hospital de Clínicas de Porto Alegre since 2015 (GPPG 2021-0512 and CAAE 53043421700005327) when the Euroflow panel was implemented. The immunophenotyping "FCS" files were reanalyzed in order to identify CD34+, CD123+, CD34+CD123+, CD34+CD38-, CD34+CD38+, CD34-CD38+ and CD34-CD38- blast subtypes. The blasts were identified as SSClowCD45-/dim and all other cell populations were excluded by CD45/CD117/HLADR/CD38 markers, mainly. Considering the hierarchical panel proposed by the Euroflow, some analyses could be done with more patients than others. That is the case for the CD34+ blasts detection, it was possible to perform this analysis for the grand majority of patients at diagnosis, first MRD (approximately 28 days after diagnosis/start of treatment) and second MRD (when available). Following, the CD34CD38 analysis could be performed with approximately half of the patients, especially the most recent ones (as the panel suffered some improvements throughout the years). The CD123+ and CD34+CD123+ subtypes were identifiable in a much smaller number of patients, due to the tube containing CD123 in the panel being the last one, meaning it is rarely needed to close the diagnosis. Also due to that, this subtype was mostly identifiable in diagnosis and not in MRDs. We will associate the presence or absence, and percentage, of each subtype to clinical outcomes such as MRD positivity and overall survival. We will also evaluate if any of the subtype's presence correlates with other known alterations in AML that define worse or better prognosis. With this work, we expect to answer which LSC-suggested phenotypes actually impact patients clinically. This approach could be beneficial for many patients, especially those living in low-income countries, that do not have access to top-of-the-line diagnosis and MRD detection methods (such as NGS) as its application can be extremely easy in places that already use Euroflow immunophenotyping protocols.

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