20.4% monocytic cells where 70% were mature monocytes (CD14+, CD300e+), 18% promonocytes (CD14+, CD300e-), and 12% monoblasts (CD14-, CD300e-), indicating acute myelomonocytic leukemia. The presence of mast cells (0.1%) was observed. Subsequently, genetic analysis and molecular biology confirmed AML with CBFB-MYH11 fusion and FLT3-D835 mutation. The treatment protocol chosen was ARA-C + Daunorubicin. One month after the beginning of induction chemotherapy, immunophenotyping of the bone marrow showed 0.6% myeloblasts with a phenotype like that found at diagnosis and 0.2% of mast cells (CD117+++), of which 53% have aberrant phenotype (CD2-/+FR, CD25+, CD64-/+, CD203c+, CD123+FR). The patient presented a serum tryptase dosage of 14.5 ug/L (reference value: 11 ug/L), and the KIT-D816 gene was not detected (performed on a blood sample). Six months after the initial diagnosis, the patient achieved remission by morphological and immunophenotyping analysis. **Discussion:** According to the latest classification of hematological tumors established by the WHO, some criteria must be met for the diagnosis of mastocytosis. Although the patient presented the presence of aberrant mast cells in the bone marrow expressing CD2 and CD25, no other criteria for the definition of mastocytosis were met. It is possible to suggest that the patient's diagnosis is systemic mastocytosis associated with a hematologic neoplasm, but more investigations will be necessary to conclude this diagnosis. Patients with mastocytosis and AHN have a worse prognosis, nevertheless, this patient achieved remission of AML with CBFB::MYH11 fusion after induction chemotherapy. **Conclusion:** It is crucial that the diagnosis and monitoring of hematologic neoplasms be carried out in a multidisciplinary manner, combining clinical and laboratory diagnostics using various techniques, particularly flow cytometry and molecular biology. In this case, these techniques were essential for diagnosing the aberrant mast cells, defining the prognosis, and monitoring minimal residual disease.

https://doi.org/10.1016/j.htct.2024.09.316

DOES PERIPHERAL BLOOD MIRROR IMMUNE TUMOR MICROENVIRONMENT? FLOW CYTOMETRY QUANTIFICATION OF PERIPHERAL BLOOD LYMPHOCYTE SUBPOPULATIONS AND OUTCOMES IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Introduction: DLBCL presents clinical, biological, and prognostic heterogeneity. The composition of the tumor TME strongly contributes for its biological diversity, being able to predict treatment response and determine prognosis. DLBCL with TME enriched in CD8+T-lymphocytes and NK cells demonstrate increased OS compared to those with TME enriched in histiocytes. Data from patients with solid tumors have established associations between absolute quantification of lymphocyte subpopulations (LS) in peripheral blood (PB) and prognosis. Therefore, it is hypothesized that the cellular constituents of PB may mirror the TME, and their quantification may constitute an easy and fast tool to access biological biomarkers predictive of therapeutic response and prognosis in malignancies. This study aims to determine the absolute quantification of LS in the PB of DLBCL patients, seeking associations with outcomes and specific clinical phenotypes. **Methods:** This study involved 54 newly-diagnosed DLBCL patients. PB collection was performed before starting any therapy, for blood count and immunophenotyping on a *BD Facs Calibur* flow cytometer. End points included OS and EFS. Survival curves were constructed using the KM method. The Mann-Whitney test and the Kruskal-Wallis test were used to establish the relationship between the lymphocyte profile, clinical variables, therapeutic response and outcomes. The Contal O'Quingley test was used to determine cut-offs for each LS capable of discriminating outcomes. Univariate analysis was performed using the Cox method and a $p\text{-}value \leq 0.05$ was considered statistically significant. **Results:** The median age at diagnosis was 54 years and 55.6% were female. Clinical stage III/IV was observed in 77.8%, 57.4% had bulky ≥ 7 cm, 63.0% had B-symptoms, and 53.7% presented IPI ≥ 3. The ORR was 80.8%. With a median follow-up of 21.3 months, the estimated 2-year OS and EFS were 73.3% and 64.7%. The median absolute values obtained for the PB lymphocyte subpopulations were: 1026 cells for CD3+ T-lymphocytes, 591 cells for CD4+ T-helper lymphocytes, 412 cells for CD8+ T-cytotoxic lymphocytes, 1.6 for CD4/CD8 ratio, 240 cells for T-reg lymphocytes, 112 cells for LG T-lymphocytes, 173 cells for NK cells, and 70 cells for B-lymphocytes. In univariate analysis, using a cut-off value = 367 CD8+T-cells/mm^3 and 141 NK-cells/mm^3, cytotoxic T-lymphodepletion was associated with decreased OS ($p = 0.005$) and EFS ($p < 0.001$), as well as NK-lymphodepletion was associated with decreased EFS ($p = 0.033$), but not with shortened OS ($p = 0.240$). DLBCL patients presenting higher NK-cell counts had lower rates of chemoresistance to the R-CHOP ($p = 0.024$), and those with higher NK-cell counts ($p = 0.033$) and higher CD8+T-lymphocyte counts ($p = 0.039$) had lower probability to develop relapse, progression or death, confirming the central role of these PB cellular constituents in anti-tumor immunity. We observed that B-cell lymphodepletion was associated with an adverse clinical phenotype, including higher frequency of bulky disease ($p = 0.009$), B-symptoms ($p < 0.001$) and advanced clinical stage ($p = 0.033$). **Conclusion:** In newly-diagnosed DLBCL, the lymphodepletion of CD8+T-cells and NK cells quantified in PB were associated with decreased OS and EFS. Similarly, peripheral T-cytotoxic and NK lymphodepletion were associated with absence of response to the R-CHOP

regimen, as well as higher probability of relapse, progression or death. Therefore, the absolute quantification of these LS in PB may be considered potential biomarkers capable of predicting response and prognosis in DLBCL. Also, we believe it may mirror the cellular composition of the immune TME.

https://doi.org/10.1016/j.htct.2024.09.317

ANALYTICAL VALIDATION OF A 10-COLOR FLOW CYTOMETRY PANEL FOR ASSESSMENT OF MEASURABLE RESIDUAL DISEASE OF ACUTE MYELOID LEUKEMIA

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The assessment of measurable residual disease (MRD) in acute myeloid leukemia (AML) by flow cytometry (FC) is challenging and requires full standardization to provide reliable results for patient management. Here is shown the analytical validation of a 10-color panel for AML MRD detection. **Material and methods:** 6 normal, 50 regenerative and 68MRD positive bone marrow (BM) samples were prepared using bulk lysis for acquisition of at least 1 million cells per tube. The panel composition was addressed to detect leukemia-associated immunophenotypes (LAIPs) and different-than-normal (DfN) immunophenotypes (clonal evolution), as well as leukemia stem cell (LSC) identification, using 5 tubes with 6 backbone markers (CD33APC/CD34PECy5.5/CD38APC-H7, CD45V500/CD117PE-Cy7,HLA-DRV450), combined with CD15/CD13/CD11b/CD7, CD2/CD56/CD123, CD97/CD99/CD54/CD244/CD18, CLL1+TIM3 /CD123/CD96/CD19, CD36/CD300e/CD45RA/CD14/CD64 in FITC/PE/AF700/BV421/BV605, respectively. Fluorescent markers were selected according to minimum spillovers between fluorescence channels and their stability after treatment. This panel was validated by comparing with our established 8-color panel. Samples were acquired in BD FACSCanto II and BD FACSLyric 10, adjusted according to EuroFlow's standard operating procedures. An unstained blank tube was run prior to sample acquisition to assess compensation and carry-over contamination was avoided by cleaning with distilled water between tubes. Infinicyt (Cytognos™) software was used for data analysis. Statistical analysis: R software (v 4.1.2) and Excel were used for statistical analysis, considering a p value $\leq$ 0.05 as significant. Bland-Altman plots and Pearson's correlation coefficient were used to assess agreement values between 10- and 8-color panels. Levey-Jennings plots, Ranksum tests, and chi-square tests were used to assess variances on the median fluorescence intensity (MFI) of the markers in CD34+ progenitor cells in normal and regenerated BM and in blast cells. The chi-square test was also used to compare the expressions of the monoclonal markers at diagnosis and during treatment. The linearity of the tests to determine the lower limit of quantification (LLOQ) was assessed using Pearson's correlation coefficient. **Results:** There was high accuracy and reproducibility comparing the performance of both panels and both flow cytometers (R2 > 0.96). The analytical sensitivity was determined by the limit of blanc (LOB) calculated using normal BM samples not stained with CD34 and CD117, limit of detection (LOD) with LOB+1.645SD; and lower limit of quantification LLOQ, using sequential dilutions of AML samples. Linearity was assessed using the results of blast cell percentages obtained from three samples diluted in duplicate, with high correlation between the measurements (R2 = 0.99; p-value < 0.001). The expression patterns of markers were evaluated according to their MFI in CD34+ progenitor cells from 6 normal and 50 regenerative BM samples compared with the MFI in blast cells from 68 MRD+ samples and there was significance difference of most values ($p < 0.05$). The hemodilution control was done by the percentages of mast cells (CD117+ bright/HLA-DR-) < 0.002% and of mature granulocytes (CD13 and CD11b bright) > 90%. **Conclusion:** The results showed a successful process of laboratory validation for AML MRD assessment, as recommended by the international consensuses (ICCS and ELN) and to authorize its use for clinical decision making.

https://doi.org/10.1016/j.htct.2024.09.318

AVALIAÇÃO DA ATIVIDADE ANTITUMORAL DO LÁTEX DE EUPHORBIA UMBELLATA

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Objetivos: Determinar a concentração inibitória mínima (IC_{50}) de linhagens tumorais hematológicas e de linfócitos humanos saudáveis (PBMCs) a partir do látex liofilizado da *Euphorbia umbellata*, bem como seu índice de seletividade (SI), a fim de avaliar se a planta em questão possui atividade antitumoral. **Material e métodos:** Células Jurkat, Raji e K-562 foram plaqueadas (1×10^5 células/poço) em triplicatas biológicas e tratadas com o látex liofilizado da *E. umbellata* (800 μg/mL; 400 μg/mL; 200 μg/mL e 100 μg/mL) por 24h. Após esse período, elas foram marcadas com 7-AAD. O mesmo protocolo foi realizado com PBMCs, a partir da coleta do sangue periférico de 6 pacientes saudáveis em tubo de heparina. As amostras foram processadas com Ficoll-Paque® (1,078 g/mL) na razão 1:2, com separação por gradiente de densidade para obtenção de PBMCs. A quantificação de morte celular em ambos os experimentos foi realizada em citômetro de fluxo BD FACS Canto II™, com aquisição e análise de 10.000 eventos por meio do software Diva 6.0 (BD Biosciences). As curvas do IC_{50} para todas as linhagens celulares testadas foram realizadas por regressão não-linear, utilizando o software Graphpad Prism® 9 (versão 8.0.2). Por fim, o SI foi obtido a partir da razão entre o IC_{50} de PBMCs e o IC_{50} das células neoplásicas. Adicionalmente, foi realizado o screening fitoquímico do látex da planta, efetuando-se os