

DEVELOPMENT OF A SPIKE-IN FLOW-CYTOMETRY METHOD FOR THE QUANTITATION OF FETAL HEMOGLOBIN (HbF) IN INDIVIDUAL RED BLOOD CELLS OF SICKLE CELL DISEASE (SCD) PATIENTS

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Aim: HbF is the most relevant modulator of the severity of SCD, inhibiting the polymerization of the mutant sickle hemoglobin (HbS). Despite its clinical relevance, a straightforward method to quantify and identify protective levels of HbF in individual red blood cells (RBCs) is still lacking. Here, we sought to develop a fluorescent-labeled RBC “Spike-In”(Spk) control (Ctr), to be mixed with patient samples, to serve as an internal reference in a novel quantitative flow-cytometry method. **Methods:** The Spk-Ctr was generated by mixing equal numbers of CFSE-labeled and fixed HbFNeg adult and HbFPoS cord blood (CB) RBCs, whose mean corpuscular HbF (MCHbF) mass was pre-calculated, based on the %HbF (HPLC-derived) and the MCH (hematology analyzer), using the formula: $MCHbF = (\%HbF \times MCH) / 100$. Spk-Ctr was mixed with a similarly-fixed Test Sample, composed of 10% HbFPoS (prepared with the same RBCs as the Spk-Ctr), permeabilized, stained with an anti-HbF PE conjugate, and acquired by a flow cytometer. The anti-HbF PE Median Intensity Fluorescence (MIF) was obtained for gated HbFPoS RBCs in both the Ctr CFSEPos and Test CFSENeg gated populations. A conversion factor (CF) was calculated by dividing the pre-calculated MCHbF by the MIF obtained for HbFPoS RBCs from the CFSEPos Spk-Ctr. By multiplying the anti-HbF PE fluorescence intensity of each RBC in the test sample by the CF, an absolute HbF/RBC values (in pg of HbF per RBC) can be obtained, which can be used to generate a distribution histogram to calculate the percentage of RBCs above a protective HbF/RBC threshold. **Results:** The Ctr CB used had 73.2% HbF and a MCH of 36.4pg, resulting in a MCHbF of 26.64pg. The anti-HbF PE MIF of the Spk-Ctr HbFPoS RBCs was 25,166a.u. (arbitrary units), resulting in a CF of 0.001058. The anti-HbF PE MIF of the HbFPoS RBCs of the Test Sample was 24,146a.u., leading to an estimated MCHbF of 25.56pg; a deviation of only 4% from the expected (known) MCHbF. In contrast, when samples were permeabilized and stained in separate tubes, this variation was 27%. **Discussion:** Recently, a work described a laborious method requiring a standard curve, generated with rare samples from subjects with hereditary persistence of HbF, and calibration beads. This method correct only for experimental variations arising from flow-cytometry acquisition and bath-to-bath antibody affinity/fluorescence differences, but not from experimental variability associated with sample handling of

the test patient samples and those used in the standard curve. Our method eliminates the complexity associated with the use of a cumbersome standard curve, as the Spike-In Ctr and patient cells are subjected to the exact same procedures (in the same tube). Thus, experimental variability (derived from differences in, pipetting, incubation time, temperature, affinity/fluorescence of antibody batches, instrument or acquisition settings, etc.) is not expected to interfere with the quantitation. **Conclusion:** Once fully developed and validated, our method may serve as an auxiliary tool for the clinical management of SCD patients, contributing to the prediction of complications (allowing preventive measures to be adopted), or allowing the monitoring of the response to therapeutic approaches based on the induction of HbF (including hydroxyurea).

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LEUCEMIA LINFOCÍTICA T “LARGE GRANULAR”. RELATO DE CASO RARO

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Objetivo: Relato de caso de Leucemia Linfocítica T “large granular”(T-LGL). **Casuística e método:** Relatamos caso pouco frequente de paciente, gênero feminino, 69 anos, que foi encaminhada para investigação diagnóstica de linfocitose. Hb 10,4g/dL, Ht 32,6%, GB 36.640/mm³, neutrófilos 2.510/mm³, linfócitos 33.440/mm³, plaquetas 220.000/mm³. Predomínio de linfócitos com características “large granular”. Imunofenotipagem por citometria de fluxo: 82,1% de células que expressam CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD16, CD56, CD57 e TCRalfa/beta e não expressam CD25, CD30, TCRgamma/delta. **Resultados e conclusões:** A leucemia de linfócitos T “large granular” está classificada na OMS 2017 entre as neoplasias de células T e NK maduras, sendo caracterizada pela proliferação clonal destas células em associação a distúrbios autoimunes tais como artrite reumatoide e presença de citopenias. Sua prevalência é rara, correspondendo a 2 – 5% das neoplasias linfoproliferativas. A presença destas células (> 2000/mm³), por um período de 6 meses ou mais, a imunofenotipagem característica e a evidência de clonalidade do gene TCR Vbeta fecham o diagnóstico. Não há distinção morfológica entre estes linfócitos anômalos e os citotóxicos normais que apresentam tamanho maior, citoplasma abundante e grânulos azurófilos. A imunofenotipagem demonstra um fenótipo pós-tímico maduro constitutivo com CD3+, TCRalfa/beta+, CD4-, CD5dim, CD8+, CD16+, CD27-, CD28-, CD45RA+ e CD57+. O caso relatado apresenta um imunofenótipo menos frequente com expressão de CD4 e CD56. Ressaltamos neste relato a raridade do caso e a importância da análise morfológica e da citometria de fluxo para a conclusão diagnóstica.

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