

understanding of the condition and improves knowledge of potential autoimmune disease risks.

<https://doi.org/10.1016/j.htct.2025.104177>

ID – 2953

**PREDICTING VASO-OCCLUSIVE CRISIS RISK IN SICKLE CELL DISEASE BY MEASURING PROTECTION, NOT JUST HbF: SINGLE-CELL QUANTIFICATION OF FETAL HEMOGLOBIN WITH AN OPTIMIZED SPIKE-IN FLOW CYTOMETRY ASSAY**

ML Arrojo<sup>a</sup>, GBC Moreto<sup>a</sup>, J Milhomens<sup>b</sup>, CCOM Bonaldo<sup>b</sup>, ACS Pinto<sup>b</sup>, SK Haddad<sup>b</sup>, RA Panepucci<sup>a</sup>

<sup>a</sup> *Laboratory of Functional Biology (LFBio), Ribeirão Preto, SP, Brazil*

<sup>b</sup> *Regional Blood Center, Ribeirão Preto, SP, Brazil*

**Introduction:** Accurate quantification of fetal hemoglobin (HbF) at the single-cell level is essential for evaluating functional protection of red blood cells (RBCs) in sickle cell disease (SCD) and for monitoring therapeutic interventions. Conventional metrics—%HbF by HPLC and F-cell analysis—lack the resolution to directly estimate mean cellular HbF (cHbF) and precisely identify functionally protected RBCs (%ProtRBC; cHbF > 10 pg), a parameter strongly associated with reduced sickling under hypoxia. **Objectives:** To develop and validate a novel flow cytometry-based assay for quantifying fetal hemoglobin content (cHbF) at the single-cell level, and to demonstrate its clinical applicability for predicting vaso-occlusive crises (VOC) in patients with sickle cell disease. **Material and methods:** RBCs from 17 HbSS patients were analyzed by quantitative flow cytometry using an optimized Spike-In assay. Spike-In calibrators were prepared by mixing normal RBCs with RBCs from a hereditary persistence of HbF (HPFH) SCD donor, generating two reference populations. In each assay,  $9 \times 10^5$  patient RBCs were mixed with  $1 \times 10^5$  fluorescently labeled Spike-In RBCs, fixed, stained with anti-HbF antibodies, acquired on a BD FACSymphony A1, and analyzed in FlowJo. Internal calibration curves converted anti-HbF fluorescence into cHbF values at the single-cell level. %ProtRBC was compared to %HbF by HPLC and classical F-cell counts. Predictive performance for vaso-occlusive crises (VOC) was evaluated by ROC analysis. Agreement between Spike-In–derived MCHbF and values calculated from HPLC-derived %HbF  $\times$  MCH was assessed by correlation, regression, and Bland–Altman analysis. Assay robustness was tested with two different HPFH Spike-In donors. **Results:** Eight patients experienced a VOC within six months. The optimized Spike-In assay yielded the highest discriminative capacity for VOC prediction (AUROC = 0.958), with 87.5% sensitivity (95% CI: 52.9–99.3%), 88.9% specificity (95% CI: 56.5–99.4%), and  $LR^+ = 7.9$  at %ProtRBC < 27.35, outperforming classical F-cell (AUROC = 0.875;  $LR^+ = 3.9$ ) and %HbF by HPLC (AUROC = 0.819;  $LR^+ = 2.6$ ). At cut-offs achieving 100% sensitivity, Spike-In retained the highest specificity (66.7%;  $LR^+ = 3.0$ ) versus F-cell

(44.4%;  $LR^+ = 1.8$ ) and HPLC (11.1%;  $LR^+ = 1.1$ ). Spike-In cHbF strongly correlated with MCHbF from HPLC  $\times$  MCH ( $r = 0.7401$ ;  $p = 0.0002$ ), with proportional agreement when regression was constrained through zero (slope = 0.8477; 95% CI: 0.7085–0.9869). Bland–Altman analysis indicated minimal bias (–0.84 pg) and narrow limits of agreement (–7.81 to 6.12 pg), confirming accuracy across the measurement range. cHbF values obtained with different HPFH Spike-In donors showed almost perfect correlation ( $r = 0.9828$ ;  $p < 0.0001$ ), demonstrating high reproducibility and portability of the method. **Discussion and conclusion:** The optimized Spike-In flow cytometry assay provides accurate, high-throughput, single-cell quantification of cHbF and superior prediction of VOC risk compared to standard methods. Its strong agreement with reference calculations, reproducibility across calibrators, and ability to resolve functional RBC heterogeneity establish it as a robust, clinically relevant biomarker platform for SCD management and for assessing pharmacological or genetic HbF-inducing therapies. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brazil (Process Number: #2022/12856-6).

<https://doi.org/10.1016/j.htct.2025.104178>

ID - 2461

**PREVALENCE AND IMPACT OF ANEMIA DUE TO HEMOLYSIS IN HIGH-PERFORMANCE ATHLETES**

GM Farias<sup>a</sup>, RS Leal<sup>b</sup>, ÉJS Freitas<sup>c,d</sup>, MO Cunha<sup>d</sup>, AGS Gbadamassi<sup>c,d</sup>, VB Lima Lobato<sup>d</sup>, MR Nascimento<sup>e</sup>, NA Fraiji<sup>e</sup>, R Ramasawmy<sup>c</sup>, JPM Neto<sup>c,d,e</sup>

<sup>a</sup> *Universidade Federal da Bahia (UFBA), Salvador, BA, Brazil*

<sup>b</sup> *Pós-Graduação em Farmácia (PPGFAR), Universidade Federal da Bahia (UFBA), Salvador, BA, Brazil*

<sup>c</sup> *Programa de Pós-Graduação em Imunologia Básica e Aplicada (PPGIBA), Universidade Federal do Amazonas (UFAM), Manaus, AM, Brazil*

<sup>d</sup> *Programa de Pós-Graduação em Ciências Farmacêuticas (PPGCF), Universidade Federal do Amazonas (UFAM), Manaus, AM, Brazil*

<sup>e</sup> *Universidade Federal de Juiz de Fora (UFJF), Juiz de Fora, MG, Brazil*

**Introduction:** Engaging in moderate to intense physical activity has been demonstrated to trigger hematological alterations, among which anemia is one of the most common and clinically relevant conditions. This condition is marked by a decrease in hemoglobin concentration within blood, leading to impairing oxygen transport and consequently diminished physical performance. Anemia in athletes, can be attributed to a variety of factors, including exercise-induced hemolysis, gastrointestinal or urinary losses, iron deficiency, and nutritional inadequacies. **Objectives:** This study aimed to review the scientific literature concerning the mechanisms,