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

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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

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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, 9×10^5 patient RBCs were mixed with 1×10^5 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 × 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 × 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).

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

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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,

diagnosis, and management of anemia in physically active individuals and athletes across various disciplines. **Material and methods:** This is a narrative literature review is based on bibliographic research conducted through the following databases PubMed, SciELO, LILACS, and Google Scholar. A comprehensive selection of articles published between 2010 and 2024 was made available selected in Portuguese, English, and Spanish. The descriptors employed included: The following terms must be defined: "sports anemia," "iron deficiency," "hemoglobin," "physical activity," and "athletic performance". **Discussion and conclusion: Results:** Iron deficiency anemia is the most prevalent form among athletes, particularly in women, long-distance runners, and endurance athletes. The primary etiological factors contributing to this condition include an augmented demand for iron, losses through perspiration, gastrointestinal microbleeding, and mechanical hemolysis resulting physical exertion, particularly in the lower limbs during running. It is important to note that sports anemia may also be confused with athlete's pseudoanemia, a physiological condition resulting from plasma volume expansion without actual reduction in red cell mass. A differential diagnosis is crucial and involves laboratory tests such as a complete blood count, serum ferritin, transferrin saturation, and hemoglobin levels. Discussion: Anemia has been demonstrated to have a detrimental effect on athletic performance and may also compromise overall health. Early identification and appropriate treatment, with a focus on nutritional strategies and supplementation, are essential for performance recovery. Interdisciplinary collaboration among health professionals, including physicians, nutritionists, and physical educators, is vital for prevention and management. Conclusion: In the domain of sports medicine, anemia is a multifactorial condition that necessitates particular consideration during clinical evaluations of athletes. A review of the extant literature indicates early interventions and individualized approaches are associated with improved physical performance enhanced health maintenance. Investments in education, periodic screening, and nutritional support are fundamental measures in the routine care of both professional and amateur athletes.

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PRINCIPAIS CAUSAS DE ATENDIMENTO MÉDICO DE PACIENTES COM ANEMIA FALCIFORME NA URGÊNCIA E EMERGÊNCIA DE UM HOSPITAL INFANTIL EM MANAUS

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Introdução: A anemia falciforme (AF) é uma hemoglobinopatia hereditária caracterizada pela substituição de um aminoácido na sexta posição da cadeia beta globina, dando origem à hemoglobina S que causa alteração morfológica dos eritrócitos. O formato característico de foice causa complicações em portadores da AF. Devido à natureza imprevisível de crises álgicas, os pacientes, especialmente crianças, frequentemente necessitam de atendimento em serviços de urgência e emergência. Entre os principais motivos para procura desses serviços estão dores intensas, episódios recorrentes de crises vaso-oclusivos, inflamação crônica, hemólise, febre persistente, icterícia e sintomas respiratórios, todos reflexos das complicações clínicas agudas da doença. **Objetivos:** Descrever os principais motivos de procura por atendimento médico em serviços de urgência e emergência por pacientes pediátricos com anemia falciforme em um hospital infantil da cidade de Manaus. **Material e métodos:** Estudo transversal retrospectivo baseado na análise de registros clínicos de pacientes com diagnóstico de anemia falciforme atendidos na urgência e emergência de um hospital pediátrico de Manaus entre os anos de 2022 e 2024. Foram incluídos registros com indicação clara do motivo clínico de atendimento. Os dados foram organizados em categorias clínicas e a frequência absoluta de cada manifestação foi contabilizada. A análise descritiva foi feita em planilha do Excel considerando os sinais e sintomas mais prevalentes. **Resultados:** As principais causas de atendimento médico foram: febre (n = 14), tosse (n = 8), dor abdominal (n = 6), diarreia (n = 6), icterícia (n = 5), dispneia e ronco no peito (n = 4), além de sintomas associados a vômito (n = 3), constipação intestinal (n = 2), coriza (n = 2), dor nos membros (n = 2). **Discussão:** Os dados observados corroboram estudos prévios que apontam a febre como o sintoma mais frequente nas crises falciformes, muitas vezes associada a infecções bacterianas graves, como pneumonia e septicemia, que constituem principais causas de mortalidade em pacientes com AF. A dor abdominal e a icterícia refletem crises hemolíticas e obstrução de pequenos vasos, especialmente no fígado e baço, comuns em crianças com doença falciforme. A alta frequência de tosse, dispneia e ronco no peito pode estar relacionada à síndrome torácica aguda, uma complicação pulmonar grave da AF. A análise também revela que sintomas menos específicos como náuseas, letargia e palidez, embora isolados, demandaram atendimento, evidenciando a complexidade clínica da doença. **Conclusão:** A febre, dor abdominal, sintomas respiratórios e gastrointestinais foram as principais causas de busca por atendimento médico de pacientes pediátricos com anemia falciforme na urgência e emergência de um hospital infantil de Manaus. A diversidade clínica observada reforça a necessidade de capacitação dos profissionais e protocolos específicos para hospitais não especializados que também atendem pacientes crônicos para o manejo rápido e eficaz das complicações da doença falciforme na infância, visando a redução da morbidade e da letalidade.

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