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CARACTERIZAÇÃO MOLECULAR DOS HAPLÓTIPOS DA HEMOGLOBINA S EM PACIENTES COM DOENÇA FALCIFORME, ATENDIDOS EM UM HOSPITAL UNIVERSITÁRIO

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Introdução: A mutação no gene β-globina, que resulta na produção da hemoglobina S (HbS), é o evento que desencadeia as obstruções vasculares, inflamação e lesões orgânicas crônicas características da anemia falciforme. A gravidade e a variabilidade fenotípica da doença estão relacionadas a fatores genéticos, especialmente aos diferentes haplótipos do gene βS-globina, os quais refletem a ancestralidade africana e influenciam os níveis de hemoglobina fetal (HbF). **Objetivos:** Identificar molecularmente os haplótipos do gene βS-globina em pacientes com anemia falciforme atendidos em um hospital universitário do Rio de Janeiro, contribuindo para a compreensão da heterogeneidade clínica da doença. **Material e métodos:** Trata-se de um estudo descritivo, de corte transversal. Foram incluídos pacientes com diagnóstico confirmado de anemia falciforme, maiores de 18 anos, em acompanhamento regular no ambulatório de hematologia. Amostras de sangue periférico foram coletadas durante a pré-consulta de rotina, após assinatura do Termo de Consentimento Livre e Esclarecido (TCLE). O material foi encaminhado ao laboratório de biologia molecular, onde foi extraído o DNA genômico pelo método de fenol-clorofórmio. A análise molecular dos haplótipos foi realizada por PCR-RFLP, utilizando a enzima DdeI e seis regiões polimórficas no cluster da β-globina. Os haplótipos foram classificados como CAR, Benin, Senegal, Camarões e Atípico, conforme padrões descritos na literatura. A pesquisa foi aprovada pelo Comitê de Ética em Pesquisa. **Resultados:** Foram analisadas 74 amostras, com identificação haplotípica conclusiva em 56 pacientes (75,7%), dos quais 32 eram homens e 24 mulheres. O haplótipo CAR foi o mais prevalente, presente em 49 amostras (87,5%), isoladamente ou em combinação. As distribuições observadas foram: 21 pacientes (37,5%) com CAR/CAR; 15 (26,8%) com CAR/Benin; 13 (23,2%) com CAR/Atípico; 4 (7,1%) com Benin/Benin; e casos isolados de Benin/Atípico, Benin/Camarões e Senegal/Atípico (cada um com 1,8%). A diversidade observada reflete a heterogeneidade genética da população brasileira, com presença significativa de haplótipos atípicos (28,6%). **Discussão e conclusão:** A predominância do haplótipo CAR corrobora dados previamente publicados sobre populações afrodescendentes brasileiras, estando associado a formas mais graves da anemia falciforme. Por outro lado, haplótipos como o Senegal são raros e geralmente associados a manifestações clínicas mais brandas. A presença expressiva de haplótipos atípicos sugere recombinações genéticas e miscigenação, características da população brasileira. Este estudo reafirma a importância da análise molecular dos haplótipos da β-globina para a compreensão da variabilidade clínica da anemia falciforme, podendo auxiliar na predição do prognóstico e na personalização do manejo terapêutico. Do ponto de vista da ancestralidade, também pode representar um valor adicional para a população afetada por essa condição.

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CHARACTERIZING THE PATHOPHYSIOLOGY OF SICKLE CELL DISEASE (SCD) EX VIVO: CREATION OF AN AUTOMATED FLUORESCENCE IMAGING ASSAY TO DYNAMICALLY EVALUATE THE IMPACT OF OXYGEN-LIMITED CONDITIONS ON RED BLOOD CELL SICKLING

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Introduction: Sickle cell disease (SCD) is a genetic blood disorder caused by a single-point mutation in the β-globin gene (c.20A>T; p.Glu6Val), leading to the production of hemoglobin S (HbS). In low-oxygen environments, HbS polymerizes and causes red blood cells (RBCs) to adopt a sickled shape, triggering vaso-occlusion, hemolysis, and systemic complications. Despite decades of research, the dynamic mechanisms driving sickling remain incompletely understood — partly due to the lack of functional tools capable of capturing these rapid and complex morphological changes in real time. **Objectives:** In this study, we present a robust, automated fluorescence imaging assay designed to monitor RBC sickling ex vivo under oxygen-restricted conditions. **Material and methods:** RBCs (5×10^4/well) obtained from SCD patients were plated on 96-well plates coated with PBS-BSA to promote adherence. A coverslip and silicone oil layer were applied to reduce oxygen diffusion. Hypoxia was chemically induced using 2% sodium metabisulfite. In parallel, we also tested an enzymatic system using glucose oxidase and catalase to evaluate a more physiological method of oxygen depletion. Images were captured every 10 seconds for 60 minutes using the ImageXpress Micro XLS system, configured with transmitted light and a DAPI filter (λ: 417–477 nm), optimizing contrast via Hb absorption at 420 nm. To analyze cell morphology, a customized pipeline in CellProfiler was combined with CellPose for AI-driven segmentation, eliminating the need for fluorescent staining. **Results:** Over 140 timepoints per cell were analyzed. A comprehensive panel of morphometric parameters—including eccentricity, solidity, compactness, and area—was extracted at each timepoint. Machine learning classification was performed using CellProfiler Analyst. RBCs were manually labeled as “Round” or “Sickled” to train a Random Forest

model, which achieved an accuracy of 97.42%. Heatmaps revealed sharp morphometric transitions during the sickling process, while individual cell tracking uncovered diverse behavioral patterns—ranging from immediate sickling to complete resistance.The assay enabled quantification of the percentage of sickled cells over time and the generation of a sickling time distribution across the entire population. These findings highlight distinct subpopulations with varying degrees of resilience to hypoxia-induced deformation. The platform also opens opportunities to explore modulatory factors, such as fetal hemoglobin (HbF). Ongoing experiments with bimodal cHbF distributions aim to determine the threshold HbF content associated with delayed or suppressed sickling. **Discussion and conclusion:** Altogether, this high-content imaging approach provides a powerful and scalable framework for dissecting the pathophysiology of SCD, offering valuable insights for personalized risk assessment and therapeutic screening. This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brazil (Process Number: #2022/12856-6).

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CLINICAL OUTCOMES AND SAFETY OF ECULIZUMAB IN ATYPICAL HEMOLYTIC UREMIC SYNDROME: A SYSTEMATIC REVIEW

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Introduction: Atypical hemolytic uremic syndrome (aHUS) is a rare, life-threatening thrombotic microangiopathy caused by dysregulated complement activation, leading to hemolysis, thrombocytopenia, and renal failure. Without timely treatment, it carries high morbidity, mortality, and risk of end-stage kidney disease. Eculizumab, a monoclonal antibody against complement C5, has transformed management by preventing complement-mediated damage, improving hematologic and renal outcomes, and reducing recurrence, including post-transplant. However, uncertainties remain about long-term efficacy, optimal treatment duration, and safety, warranting comprehensive evidence synthesis. **Objectives:** To systematically review and synthesize evidence on the clinical outcomes of eculizumab in patients with atypical hemolytic uremic syndrome (aHUS) and related thrombotic microangiopathies, focusing on its effects on renal function, hematologic parameters, recurrence and rejection rates post-transplant, outcomes in specific patient populations, and safety profile. **Material and methods:** A systematic review was conducted in accordance with PRISMA guidelines. PubMed was searched for studies published between 2017 and 2025 using terms related to eculizumab, atypical hemolytic uremic syndrome, and clinical outcomes, including systematic reviews and meta-analyses. After removing duplicates, studies were screened by title, abstract, and full text. Nine articles met the eligibility criteria and were included in the qualitative synthesis. **Discussion and conclusion:** Across systematic reviews, eculizumab, a C5 inhibitor, demonstrated consistently favorable outcomes in atypical hemolytic uremic syndrome (aHUS) and related thrombotic microangiopathies (TMA). In a Cochrane review (n = 137), no deaths occurred after 26 weeks of therapy. Renal function improved significantly, including in post-transplant patients, with higher GFR (+59.57 mL/min), lower serum creatinine (−126.93 μmol/L), and markedly reduced dialysis needs (OR = 0.13); similar stabilization or recovery was observed in drug-associated TMA cases. Hematologic recovery was robust, with complete TMA response in 60–65% at follow-up, platelet count increases (+163.42 × 10^9/L), and LDH reduction (−336.61 U/L). In post-transplant settings, recurrence (OR = 0.05) and rejection (OR = 0.09) risks were substantially reduced. Pediatric data (n = 940; 682 on eculizumab) showed improvement in most cases and 1.6% mortality. Pregnancy-associated aHUS remission rates were higher with treatment (88% vs 57%), and 93% of SLE-associated TMA patients improved, with nearly half discontinuing therapy without relapse. Serious adverse events occurred in 42%, including rare meningococcal infections. Overall, eculizumab yields significant renal and hematologic benefits, lowers recurrence and rejection post-transplant, and is effective across diverse patient groups, though vigilance for adverse events remains essential. Eculizumab is a highly effective therapy for aHUS, producing significant improvements in renal function, hematologic parameters, and reducing dialysis needs. It lowers recurrence and rejection rates post-transplant and demonstrates efficacy across pediatric, pregnancy-associated, and autoimmune-related TMAs. While serious adverse events, including rare meningococcal infections, can occur, the overall benefits in survival, remission, and organ recovery strongly support its use, with further studies needed to optimize long-term management.

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CO-HERANÇA DA ANEMIA FALCIFORME COM A DELEÇÃO ALFA -3.7 KB E A VARIANTE DE GLOBINA ALFA HB STANLEYVILLE-II: RELATO DE CASO

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