

clínicos como laboratoriais foram obtidos através de fichas eletrônicas, e para avaliação dos parâmetros clínicos, foram utilizadas as variáveis: crise vaso-oclusiva (VOC) no último ano, histórico de AVC e/ou TEV, e osteonecrose (ON). A expressão em monócitos CD41+CLEC2+ teve diferença entre os grupos, teste Kruskal-Wallis [$X^2(2) = 19,9$; $p < 0,0001$], os pacientes DF-SS e DF-SC tinham expressão aumentada quando comparado aos indivíduos saudáveis ($p < 0,0001$; $p = 0,01$, respectivamente), não observou diferença entre os pacientes DF-SS e DF-SC ($p = 0,99$, Bonferroni, post-hoc). Na expressão de granulócitos CD41+CLEC2+ também observou diferença entre os grupos [$X^2(2) = 11,6$; $p = 0,003$], os pacientes DF-SS tinham um aumento da porcentagem quando equiparados aos indivíduos saudáveis ($p = 0,002$), não houve diferença entre DF-SS e DF-SC ($p = 0,99$), e DF-SC e indivíduos saudáveis ($p = 0,07$). Em relação aos parâmetros clínicos, os granulócitos CD41+CLEC2+ estavam elevados no grupo com ON quando comparados ao grupo sem ON ($p = 0,02$), bem como tinha uma tendência de estar elevado nos pacientes com VOC ($p = 0,06$). A expressão de monócitos CD41+CLEC2+ estava elevada nos pacientes que tiveram AVC/TEV quando equiparados aos que não tiveram AVC/TEV ($p = 0,04$). Na avaliação dos parâmetros laboratoriais, a partir da variável monócitos CD41+CLEC2+ observou correlação com hemoglobina ($R = -0,507$), reticulócitos ($R = 0,445$), RDW ($R = 0,599$), D dímero ($R = 0,643$), FvW: Antígeno ($R = 0,495$), granulócitos CD41+CLEC2+ ($R = 0,850$), monócitos CLEC2+CD62P+ ($R = 0,760$) e monócitos PDPN+ ($R = 0,494$). Atualmente, poucos estudos relatam a avaliação de AgLP na DF, apesar da associação dos AgLP com a fisiopatologia da VOC ter sido demonstrada em modelos animais, tais achados não foram explorados em estudos clínicos. Além disso, nenhum trabalho relatou a associação dos AgLP com a via PDPN/CLEC-2. Assim, sugere-se que os AgLP que expressam CLEC-2 possa ser um mecanismo importante associado a hipercoagulabilidade na DF, uma vez que correlacionou com marcadores clínicos e laboratoriais.

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THE INFLAMMASOME DRIVES MICROVASCULAR IMPAIRMENT IN MOUSE MODELS OF INTRAVASCULAR HEMOLYSIS

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Introduction: Intravascular hemolysis (IVH) results in the release of damage-associated molecular patterns (DAMPs) into the circulation, particularly hemoglobin (Hb) and heme, which can trigger NLRP3 inflammasome activation and a sterile inflammatory response. Complications of hemolytic diseases include thrombosis, inflammation, and organ damage. In sickle cell disease (SCD), IVH can lead to vaso-occlusive crises and ischemic complications such as cutaneous leg ulcers, acute chest syndrome, and multiorgan infarction. **Aim:** To investigate the in vivo effects of inflammasome formation on microvascular function and its role in the pathophysiology of hemolytic conditions. **Methods:** We utilized two murine

models to simulate intravascular hemolysis: an acute model where C57BL6 mice received osmotic stress (150 μ l sterile water, i.v. -HEM); and a chronic model induced by repeated low doses of phenylhydrazine (10 mg/kg, i.p. -CHEM). Control mice received saline under similar conditions. Microvascular dysfunction was assessed by laser Doppler fluxometry (LDF) in the skin of the pelvis and by intravital microscopy of the cremaster muscle. Inflammasome activation was evaluated via flow cytometry measurement of caspase-1 (casp-1) activity, measurement of interleukin (IL)-1 β and IL-18 release by ELISA, and NLRP3 protein expression in the liver using western blot. **Results and discussion:** Our findings show that acute hemolysis immediately increased plasma cell-free Hb and heme levels in HEM mice, along with decreased Hb scavenger haptoglobin (Hp). CHEM mice also displayed a significant depletion of hemopexin (Hx), indicating the persistent removal of this heme-neutralizing protein. These results confirm that these mouse models effectively mimic acute and chronic hemolytic stress. Acute IVH resulted in microvascular dysfunction characterized by a vaso-occlusive-like process, as demonstrated by an increase in rolling, adherent, and extravasated leukocytes. The blood flow velocity of HEM mice was reduced in the microcirculation, resulting in impaired tissue blood perfusion. These findings were accompanied by increased casp-1 activity in peripheral monocytes and elevated levels of IL-1 β and IL-18, all hallmarks of inflammasome formation, suggesting that inflammasome assembly during hemolytic stress contributes to tissue microcirculation dysfunction. While acute hemolysis caused microvascular defects and impaired blood perfusion in mice, chronic hemolysis significantly affected the liver, leading to an increase in the macrophage population, which displayed elevated active casp-1 and increased NLRP3 protein expression. Next, we investigated whether the NLRP3 inflammasome contributes to hemolysis-induced microvascular pathology. As expected, casp-1 activation was reduced by the NLRP3 inhibitor, MCC950, in both the neutrophils and monocytes of HEM mice. Moreover, NLRP3 inflammasome inhibition reduced leukocyte recruitment to the venule walls of HEM mice, indicating an improvement in hemolysis-induced microvascular dysfunction. This was further corroborated by LDF, which showed improved blood perfusion in the skin of MCC950-treated HEM mice. **Conclusion:** These results demonstrate that NLRP3 inflammasome assembly, in response to hemolysis, compromises microvascular function by modulating leukocyte adhesion to the endothelium and impairing blood flow to tissues, which could contribute to clinical complications in hemolytic disorders such as SCD.

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ANEMIA HEMOLÍTICA POR DEFICIÊNCIA DE FOSFATO ISOMERASE: UM RELATO DE CASO

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