IDENTIFICAÇÃO LABORATORIAL DO ANTICOAGULANTE LÚPICO (AL) UTILIZANDO DIFERENTES REAGENTES DE TEMPO DE TROMBOPLASTINA PARCIAL ATIVADA (TTPA)

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Introdução: A detecção do Anticoagulante Lúpico (AL) deve seguir as diretrizes da ISTH que recomenda a avaliação em três fases: triagem, mistura e confirmatória. O ensaio baseado no TTPa deve ser realizado preferencialmente com sílica como ativador e sua etapa confirmatória com uma maior concentração de fosfolípides (bicamada ou hexagonal). No Brasil, as principais plataformas para a detecção de AL são Instrumentation Laboratory (IL), Siemens Healthcare Diagnostics e Diagnostica Stago. Apesar das três seguirem as etapas recomendadas, há diferenças significativas nos seus reagentes, particularmente o reagente da Siemens cuja composição não é citada na recomendação da ISTH. Faltam estudos comparativos sobre a acurácia desses testes que auxiliem na escolha da plataforma a ser utilizada. **Objetivo:** Comparar a sensibilidade e especificidade de três tipos diferentes de reagentes TTPa disponíveis no mercado brasileiro para a detecção do AL, sendo que em um deles o tipo de reagente confirmatório não é o preferencialmente indicado pela ISTH. **Metodologia:** Amostras de plasma para investigação de AL foram obtidas entre fevereiro e junho de 2024, nos Laboratórios de Hemostasia do Serviço de Hematologia e Terapia Celular e do Instituto do Coração do HCFMUSP. As amostras foram testadas usando PTT-LA/Staclot LA (Diagnostica Stago), Dade Actin FSL/FS (*Siemens Healthineers*) e *Silica Clotting Time* (IL) e analisadas com testes estatísticos de sensibilidade, especificidade e teste ponderado de Kappa. Foram consideradas verdadeiro-positivas as amostras com positividade em pelo menos duas plataformas. **Resultados:** Foram testadas 163 amostras, a maioria de mulheres (81%) com uma idade mediana de 41 anos (IIQ 31–50). Todas tinham indicação clínica para a pesquisa laboratorial de AL. Entre as amostras, 57 tinham história de trombose prévia e 45 em uso anticoagulantes. A plataforma Stago detectou 9 amostras positivas, enquanto a Siemens detectou 12 e a IL 32. A comparação entre as plataformas demonstrou uma concordância substancial entre Siemens e Stago ($\kappa = 0{,}746$ e 97% de concordância), enquanto a IL apresentou uma concordância fraca tanto com a Stago ($\kappa = 0{,}386$ e 86% de concordância) quanto com a Siemens ($\kappa = 0{,}287$ e 83% de concordância). A plataforma Stago apresentou a maior sensibilidade (100%) e especificidade (100%) dentre as três. A IL também demonstrou uma sensibilidade alta, de 100%, mas com uma especificidade de 85%. Siemens teve uma sensibilidade de 89% e uma especificidade de 97%. Com o objetivo de melhorar a especificidade do teste da plataforma IL, modificamos o reagente de triagem adicionando uma concentração maior de fosfolípides. Dos pacientes AL positivos pela plataforma da IL, apenas 43% permaneceram positivos após essa modificação o que aumentou a especificidade do teste para 97%. Das 12 amostras negativas no teste modificado da plataforma IL, 11 já eram negativas em pelo menos uma das outras plataformas. **Discussão/conclusão:** Observamos uma boa concordância entre os resultados das plataformas Stago e Siemens, Entretanto, a plataforma Siemens apresentou menor sensibilidade e a IL menor especificidade. O aumento da concentração de fosfolípides no teste de triagem melhorou a especificidade da plataforma IL. Além disso, o teste da Siemens mostrou boa performance, quando comparado aos demais, apesar de não utilizar os reagentes preferenciais indicados pela ISTH.

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DROPLET MICROFLUIDICS FOR DETECTING HYPER-REACTIVE PLATELETS

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Introduction: Cardiovascular Diseases (CVDs) are the main cause of morbidity and mortality globally. Of these, elevated platelet reactivity is a common cause of heart attacks and strokes. Platelet function assay typically measures platelet bulk response to an agonist, potentially overlooking hyper-reactive platelets, which can drive thrombus formation and present a risk of heart attacks and strokes. To overcome this challenge, we have combined droplet microfluidics with flow cytometry for high-throughput single-platelet function analysis to identify hyper-reactive platelets. Here we evaluate the technology's suitability for characterising models of elevated platelet reactivity and extend this to the measurement of platelets from at-risk patients as a first step towards assessing the candidacy of the technology for predicting thrombosis risk. **Objectives:** Use the droplet microfluidics and flow cytometry method to identify hyper-reactive platelets, enabling us to understand the role of hyper-reactive platelets in health, in models of disease (priming) and clinical samples. Evaluate the candidacy of the method for predicting thrombosis risk in at-risk clinical cohorts (chest infection and fractured neck of femur). **Material and methods:** Platelet-Rich Plasma (PRP) was obtained from healthy donors (IRAS: 320501/REC: 22/LO/0801) by centrifugation. Droplets were generated containing PRP, with or without priming with Thrombopoietin (TPO), as well as convulxin concentrations as the agonist. Platelets were

released from the droplets and fixed for cytometry analysis using P-selectin and PAC-1 end-points. For the small clinical study (chest infection and fractured neck of femur), ethics approvals were obtained (IRAS: 321105 /REC: 14/SC/0211) and droplets were generated only containing PRP and analyzed as described above. **Results and discussion:** We detected hyper-reactive platelets by comparing the responses of single platelets confined in droplets (single) with bulk platelet responses (collective) experiments. In bulk experiments, these hyper-reactive platelets cooperate by paracrine signalling to increase system-level sensitivity, highlighting the importance of hyper-reactive platelets. TPO priming increased platelet reactivity to ADP, but not to convulxin, implying the coupling of priming pathways with P2Y12 receptor activation by ADP, but not GPVI receptor activation by convulxin. By test-driving droplet cytometry with at-risk patient cohorts, we found one chest infection patient with elevated platelet reactivity and one fractured neck of the femur patient with increased platelet sensitivity. Bulk cytometry did not detect these characteristics, indicating that paracrine signalling masks hyper-reactive and hyper-sensitive platelets. **Conclusion:** Droplet cytometry can be used to detect hyper-reactive platelets by excluding paracrine signalling and warrants further investigation to assess the potential for predicting thrombosis risk in the clinic.

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EXPLORATION OF PURINERGIC SIGNALING PATHWAYS AS A MECHANISM OF PLATELET ACTIVATION IN ANTIPHOSPHOLIPID SYNDROME

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Background: We have previously demonstrated hyperreactivity to ADP, overexpression of the P2Y12 receptor, and reduced intracellular levels of cAMP and cGMP in platelets from Antiphospholipid Syndrome (APS) patients, suggestive of purinergic signaling activation. A possible explanation could be impaired adenosine pathways, since adenosine limits platelet activation by increasing intracellular cAMP via its A2A and A2B receptors. **Aims:** To evaluate the action of Adenosine Receptors (AR) on platelet activity in thrombotic primary APS (t-PAPS). **Methods:** t-PAPS and healthy volunteers were included. Platelet-rich plasma was used to evaluate platelet aggregation (induced by ADP 3 uM and collagen 1 μg/mL). Washed platelets (1X10^8 platelets/mL) were used to assess platelet activity (CD62P expression and CD41/CD61) induced by thrombin 0.005U in flow cytometry. CD62P or P-selectin, plays a key role in cell adhesion during neutrophil rolling and interactions between platelets and neutrophils and monocytes. CD41/CD61 or PAC-1, is a ligand-binding site for fibrinogen, von Willebrand factor, fibronectin, and vitronectin. Adenosine, NECA (stable adenosine analogue), CGS21680 (A2A receptor agonist) were used to evaluate adenosine pathway signaling. These are potent inhibitors of ADP-induced platelet aggregation and enhance the effects of P2Y12 antagonists. **Results:** A total of 53 t-APS and 63 controls were included, women were 74% of t-PAPS and 71% of controls. The median age was 46 years (IQR 37–55) for t-PAPS and 43 years (IQR 32–51) for controls ($p = 0.16$). Notably, 25% of t-PAPS patients were triple-positive for antiphospholipid antibodies. Adenosine 1 μM inhibited ADP-induced aggregation by 19% (IQR 7%–48%) in t-PAPS and 38% (IQR 17%–61%; $p = 0.04$) in controls. Adenosine 10 μM inhibited 75% (IQR 64%–88%) of ADP-induced aggregation in t-PAPS and 84% (IQR 76%–92%; $p = 0.01$) in controls. Adenosine 10 μM also inhibited collagen-induced platelet aggregation by 91% (IQR 73%–94%) in t-PAPS and 94% (IQR 88%–98%; $p = 0.03$) in controls. CGS21680 1 μM inhibited ADP-induced aggregation by 50% (IQR 28%–73%) in t-PAPS and 67% (IQR 41%–83%; $p = 0.03$) in controls. CGS21680 10 μM inhibited ADP-induced aggregation by 79% (IQR 69%–85%) in t-PAPS and 88% (IQR 79%–93%; $p = 0.02$) in controls. In flow cytometry analysis, CGS21680 at 10 μM inhibited P-selectin expression by 12% (IQR 3%–18%) in t-PAPS and 18% (IQR 9%–30%, $p = 0.05$) in controls. NECA at 10 μM inhibited P-selectin expression by 13% (IQR 6%–24%) in t-PAPS and 22% (IQR 11%–35%; $p = 0.04$) in controls. CGS21680 at 10 μM also inhibited PAC-1 expression by 25% (IQR 17%-37%) in t-PAPS and 30% (IQR 23%–52%; $p = 0.02$) in controls. NECA at 10 μM inhibited PAC-1 expression by 27% (IQR 18%–42%) in t-PAPS and 35% (IQR 27%–58%; $p = 0.01$) in controls. **Discussion:** Platelets from patients with t-PAPS exhibited resistance to the inhibition effects of adenosine, CGS21680, and NECA. This resistance was observed in two distinct functional assays: platelet aggregation and cytometry. This observation raises the hypothesis that the activation of purinergic signaling in platelets from APS patients may be explained by a reduction in the regulatory activity of adenosine pathway. **Conclusion:** Platelets from patients with t-PAPS display resistance to platelet inhibition via the adenosine pathway. The stimulation of adenosine pathway may form a potential therapeutic option to control hypercoagulability in APS.

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RISCO TROMBÓTICO E MORTALIDADE EM PACIENTES COM SUSPEITA DE TROMBOCITOPENIA INDUZIDA PELA HEPARINA (HIT)

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Objetivo: Avaliar uma coorte de pacientes com suspeita de HIT, comparando ocorrência de trombose, sangramento ou morte entre pacientes com confirmação ou não diagnóstica.