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.