PFO-LIKE INTRACARDIAC FLOW IS SUFFICIENT TO INDUCE A PROTHROMBOTIC PLATELET PHENOTYPE: EVIDENCE FROM A MICROFLUIDIC MODEL OF PATENT FORAMEN OVALE

M. Conti[1], V. Guadagno[2], D. Trabattoni[1], A. Becchetti[1], F. Morelli[2], L. Fusini[1], M. Brambilla[1], S. Bozzi[2], A. Redaelli[2], M. Camera[1|3]

[1]Centro Cardiologico Monzino IRCCS, Milano, Italy; [2]Politecnico di Milano, Department of Electronics, Informatics and Bioengineering (DEIB), Milano, Italy; [3]Università degli Studi di Milano, Department of Pharmaceutical Sciences, Milano, Italy

Background and Aims. Patent foramen ovale (PFO) is increasingly recognized as a clinically relevant condition associated with migraine with aura and thromboembolic complications. Beyond paradoxical embolism, growing evidence suggests that PFO patients display a prothrombotic phenotype characterized by platelet activation, oxidative stress, and increased exposure of platelet-associated Tissue Factor (TF), a key initiator of thrombin generation. However, the mechanisms underlying this phenotype remain poorly understood. It is unclear whether platelet activation is primarily driven by circulating biochemical mediators or by the abnormal intracardiac flow dynamics generated by blood passage through the PFO tunnel. Computational fluid dynamics simulations indicate that blood cells traversing PFO-like geometries are exposed to abnormal shear gradients and mechanical stress, potentially contributing to platelet activation. We aimed to investigate whether PFO-like hemodynamics is sufficient to induce platelet activation and to reproduce the prothrombotic platelet phenotype observed in patients with PFO.

Methods. Platelet phenotype was first analyzed in PFO patients before percutaneous closure and after a 6-month follow-up, and compared with healthy subjects (HS) by flow cytometry. Platelet activation was assessed by measuring TF exposure and reactive oxygen species (ROS) generation. To investigate the mechanistic contribution of altered flow, a microfluidic platform reproducing the geometry and hemodynamic conditions of a PFO tunnel was developed based on computational fluid dynamics simulations (Figure). Whole blood from HS was perfused through the PFO-mimicking chip or through a linear physiological-flow chip used as control. Platelet TF expression, P-selectin exposure, activated GPIIbIIIa (aGPIIbIIIa) and ROS generation were analyzed by flow cytometry. In selected experiments, blood was pre-incubated with aspirin or a P2Y12 inhibitor (ARMX) before perfusion.

Results. PFO patients showed a significantly enhanced platelet prothrombotic phenotype, characterized by increased TF-positive platelets and ROS production compared with HS, which significantly decreased after PFO closure, supporting a direct link between PFO and systemic platelet activation. Perfusion of whole blood from HS through the PFO-mimicking chip was sufficient to reproduce this phenotype ex vivo. Passage through the PFO chip induced a significant increase in platelet TF expression, ROS production and P-selectin exposure (2-fold vs linear chip), whereas linear flow conditions did not significantly modify these parameters. In contrast, aGPIIbIIIa showed only a non-significant increase. Pre-incubation with the ARMX significantly reduced TF expression and ROS generation induced by PFO-like flow, whereas aspirin mainly reduced ROS.

Conclusions. These findings provide mechanistic evidence that abnormal PFO-associated intracardiac flow is sufficient, per se, to induce a prothrombotic platelet phenotype characterized by TF exposure. The ability of the microfluidic PFO model to recapitulate the platelet phenotype observed in vivo supports a direct causal role of hemodynamic stress in PFO-related thromboinflammation. This model may represent a valuable translational platform to dissect mechanisms of platelet activation in PFO and to test targeted antithrombotic strategies. Funding: M6C2I2.1_PNRR_NEXT_GENERATION_EU, PNRR-MAD-2022-12376277, CUP-B43C22001110008 Clinical trial: NCT06046508

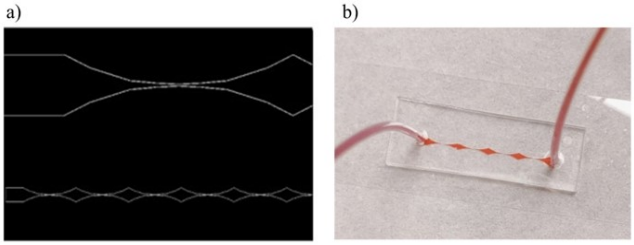


Figure. Microfluidic chip reproducing PFO-like geometries. a) AutoCAD software imagine used for project design (single and multiple feature); b) chip in use where blood flow can be observed.