




Biologia vascolare

# PERSISTENT ENDOTHELIAL THROMBOINFLAMMATORY PHENOTYPE IN PATENT FORAMEN OVALE: EVIDENCE FROM PATIENT-DERIVED ENDOTHELIAL COLONY-FORMING CELLS

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**Background and Aims.** Patent foramen ovale (PFO) is increasingly recognized as a condition associated with thromboinflammatory milieu, particularly in patients with migraine with aura. Beyond platelet activation, endothelial dysfunction may represent a contributor to this prothrombotic state. Endothelial colony-forming cells (ECFCs), as patient-derived endothelial progenitors retaining donor-specific characteristics, provide a valuable model to investigate endothelial thrombogenicity and vascular adaptation in PFO. We aimed to characterize the endothelial procoagulant phenotype, response to physiological shear stress and transcriptional changes associated with PFO closure in ECFCs from PFO patients.

**Methods.** ECFCs were isolated from peripheral blood of 35 PFO patients before closure (T0) and 6 months after closure (T1), and from 25 healthy subjects (HS). Intracellular Tissue Factor (TF) and Tissue Factor Pathway Inhibitor (TFPI) expression were evaluated by flow cytometry and ELISA. Functional procoagulant activity was assessed by CAT. To investigate endothelial mechanoadaptation, ECFCs were exposed to high laminar shear stress (HLSS), and the expression of genes involved in endothelial homeostasis, vascular tone, inflammation, and coagulation (e.g. KLF2, KLF4, NOS3, EDN1, HMGB1, and TF) was quantified. RNA sequencing was performed to compare endothelial profiles before and after PFO closure.

**Results.** ECFCs derived from PFO patients displayed a clear procoagulant phenotype. TF expression was significantly increased at baseline compared with HS and decreased after PFO closure, supporting a direct association between PFO and endothelial prothrombotic activation. Consistently, the TF/TFPI balance was shifted toward a procoagulant state in patients, with an increased TF/TFPI ratio and enhanced thrombin-generating capacity. A greater proportion of ECFC lines from PFO patients generated measurable thrombin compared with HS, and this functional procoagulant tendency persisted after closure, suggesting only partial restoration of endothelial hemostatic balance. Under HLSS, healthy ECFCs activated a physiological mechanoprotective program characterized by induction of flow-responsive genes. In contrast, ECFCs from PFO patients showed an impaired adaptive response, with reduced induction of shear-responsive protective mediators, particularly KLF4, and persistence of pro-inflammatory and procoagulant signals, including TF and HMGB1. Transcriptomic analysis revealed that PFO closure induced partial endothelial reprogramming, with downregulation of pathways associated with coagulation, platelet interaction, and inflammatory activation, together with upregulation of pathways involved in vascular repair, tissue remodeling, and immune regulation.

**Conclusions.** PFO is associated with a distinct endothelial thromboinflammatory phenotype characterized by enhanced TF-driven procoagulant activity and impaired adaptation to physiological shear stress. PFO closure partially restores endothelial homeostasis, but residual prothrombotic features persist, suggesting that endothelial dysfunction may represent a component of the thromboinflammatory burden associated with PFO. These findings identify the endothelium as an active contributor to PFO-associated thrombogenicity and a potential target for biological stratification and therapeutic intervention. Funding: M6C2I2.1_PNRR_NEXT_GENERATION_EU, PNRR-MAD-2022-12376277, CUP-B43C22001110008 Clinical trial: NCT06046508