



TROMBOEMBOLISMO VENOSO: ASPETTI CLINICI E
DIAGNOSTICI

ROLE OF CONSTITUTIVE ENDOTHELIAL DYSFUNCTION IN THE PATHOGENESIS OF UNPROVOKED VENOUS THROMBOEMBOLISM: CHARACTERIZATION OF ENDOTHELIAL COLONY-FORMING CELLS AND ASSOCIATION WITH RISK OF RECURRENCE

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Background. Venous thromboembolism (VTE) can be associated with provoking factors, either major (defining provoked events, pVTE) or minor (defining weakly provoked events, wpVTE). Nonetheless, up to 40-50 % of VTE cases occur in absence of known triggering factors (unprovoked events, uVTE) and show high risk of recurrence. Since endothelial dysfunction (ED) plays a crucial role in triggering thrombus formation, possible presence of constitutive endothelial alterations deserves to be investigated in uVTE patients. In this regard, endothelial colony-forming cells (ECFCs) represent a valuable tool to study patient-specific endothelium.

Aims. To investigate the presence of alterations in ECFCs of VTE patients, thus identifying endothelium perturbation(s) involved in uVTE pathogenesis and recurrence risk.

Methods. ECFCs were isolated from 123 patients- classified as uVTE (n=28), wpVTE (n=58), pVTE (n=37) - and 58 matched healthy donors (HDs). Three months after the acute event, patients were evaluated for clinical predictors of VTE recurrence and underwent peripheral blood sampling for ECFC isolation. ED was assessed by analyzing (i) platelet deposition and fibrin formation through thrombogenesis assay; (ii) thrombin formation through thrombin generation assay (TGA) on ECFCs; (iii) ECFC expression of adhesion molecules and pro-coagulant and anti-coagulant proteins; (iv) soluble forms of adhesion molecules in ECFC supernatant and plasma. The project was funded by the Italian Ministry of Health (GR-2018-12365520).

Results. In thrombogenesis assays, uVTE-ECFCs supported increased platelet deposition compared with HD ($p<0.05$), with no differences in fibrin formation. This pro-adhesive phenotype was associated with increased ECFC expression of ICAM-1 and PECAM-1 versus HD ($p<0.05$) and higher VCAM-1 versus HD and provoked VTE ($p<0.01$), paralleled by increased sICAM-1 and sPECAM-1 in ECFC supernatants and selective plasma sICAM-1 increase in uVTE. TGA showed faster and greater thrombin generation in uVTE versus HD and pVTE ($p<0.05-0.01$). Consistently, uVTE-ECFCs displayed increased tissue factor (TF) and reduced thrombomodulin (TM) expression, resulting in a higher TF/TM ratio ($p<0.01$). Overall, ED-related features were predominantly observed in the uVTE group, which also clustered with a higher risk of VTE recurrence based on clinical predictors (Table).

Conclusions. uVTE patients display a distinct ED phenotype and the highest recurrence risk, suggesting that ED may contribute to uVTE pathogenesis and represent a relevant feature for clinical risk stratification.

Table. Clinical predictors of recurrent VTE across study population subgroups

Validated predictor	Threshold for defining High recurrence risk (HRR) patients	% of patients identified as HRR			uVTE vs wpVTE	uVTE vs pVTE	wpVTE vs pVTE
		uVTE	wpVTE	pVTE			
Post-thrombotic syndrome	Villalta score ≥ 5	4.53% (1/22)	16.67% (8/48)	7.41% (2/27)	ns	ns	ns
D-dimer	Plasmatic levels $> 250 \mu\text{g/ml}^*$	32.14% (9/28)	29.09% (16/55)	44.44% (12/27)	ns	ns	ns
Residual Obstruction (RO)	Presence of venous or pulmonary vascular obstruction	74.1% (20/27)	48.3% (28/58)	45.9% (12/27)	$p<0.05$	$p<0.05$	ns
VTE Recurrence - clinical prediction models							
"Men continue and HERDOO2"	Men; Women with score ≥ 2	89.28% (25/28)	63.79% (37/58)	54.05% (14/27)	$p<0.05$	$p<0.01$	ns
DASH score	Score ≥ 2	42.86% (12/28)	18.96% (11/58)	32.43% (12/27)	$p<0.01$	ns	ns
Vienna Model	Recurrence risk $> 5\%$ at 12 months	28.57% (8/28)	26.32% (15/57)	18.92% (7/27)	ns	ns	ns

Statistical significance was calculated using χ^2 test

*measured at 3 months on anticoagulant therapy; HERDOO2: Hyperpigmentation, Edema, Redness, D-dimer, Obesity, Older age; DASH: D-dimer, Age, Sex, Hormone use