

Impact of soluble thrombomodulin and activated protein C on dynamic hemostatic function in trauma: a focus on thrombin generation and clot lysis

Nicola S. Curry,^{1,2} Jeries Abu-Hanna,^{1,2} Gael B. Morrow,¹⁻³ Robin Choudhury^{2,4} and Michael Laffan^{1,5}

¹Oxford Haemophilia & Thrombosis Centre, Oxford University Hospitals NHS Foundation Trust, Oxford; ²Radcliffe Department of Medicine, Oxford University, Oxford; ³School of Pharmacy and Life Sciences, Robert Gordon University, Aberdeen; ⁴Department of Cardiovascular Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford and ⁵Department of Immunology and Inflammation, Faculty of Medicine, Imperial College London, London, UK


Correspondence: N.S. Curry
nicola.curry@ouh.nhs.uk

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Supplementary Data.

Table S1. Interaction of added Tissue Factor and soluble thrombomodulin in healthy volunteers.

	Innovin* (dilution)	Concentration of added soluble thrombomodulin (ng/mL)			
		0 TM	4TM	8TM	16 TM
Lagtime (mins)	Neat	7.2 (0.27)	7.1 (0.17)	7.1 (0.16)	7.2 (0.27)
	1 in 2	8.7 (0.5)	7.3 (0.17)	8.7 (0.31)	5.3 (0.16)
	1 in 4	8.8 (0.17)	9 (0.41)	8.3 (0.28)	8.9 (0.14)
	No innovin	13.1 (0.6)	15.6 (1.52)	13.2 (1.21)	14.7 (2.01)
ETP (nM/min)	Neat	2588 (22.7)	2550 (71.8)	2401 (44.9)	2321 (84.8)
	1 in 2	2555 (14.6)	1793 (76.3)	1931 (105.9)	2034 (156.8)
	1 in 4	2388 (54.4)	2276 (39.3)	2371 (103.9)	2274 (84.6)
	No innovin	982 (29.5)	874 (156.3)	939 (127.2)	614 (43.1)
Peak height (nM)	Neat	194 (3.99)	191 (0.69)	191 (1.81)	195 (3.75)
	1 in 2	172 (2.2)	161 (8.48)	180 (3.91)	253 (23.2)
	1 in 4	156 (3.3)	181 (21.4)	176 (7.4)	176 (4.4)
	No innovin	73 (10.6)	84 (19.5)	78 (5.3)	58 (3.3)
Time to Peak (mins)	Neat	13.3 (0.47)	13.5 (0.17)	13.5 (0.16)	13.0 (0.31)
	1 in 2	19.2 (0.57)	14.4 (0.17)	17.5 (0.16)	9.8 (0.31)
	1 in 4	17.0 (0.5)	16.5 (1.17)	15.5 (0.17)	16.3 (0)
	No innovin	20.2 (0.67)	22.2 (1.36)	18.9 (1.0)	58.2 (1.0)
Start tail (mins)	Neat	40.6 (0.79)	40.4 (0.33)	41 (0.27)	38.8 (0.83)
	1 in 2	47.4 (1.5)	37.6 (0.17)	43.6 (0.94)	29.1 (2.75)
	1 in 4	44.8 (0.33)	43.9 (1.5)	44.1 (0.5)	41.3 (0.17)
	No innovin	48.0 (1.28)	46.0 (3.2)	41.0 (0.98)	43.2 (1.12)

Serial dilutions were performed on Innovin®(Dade®), with a starting dilution of 1 in 17,000 (denoted as 'neat' concentration of Innovin in the table, determined to be a concentration of 1pM TF).

Data are mean (n = 20) and standard deviation.

Key: ETP – endogenous thrombin potential.

Table S2. Effect of soluble TM concentrations on thrombin generation in healthy volunteers.

	Concentration of added soluble thrombomodulin (ng/mL)					One way ANOVA p value
	0 TM	4 TM	8 TM	16 TM	64 TM	
Lagtime (min)	12.5 (2.5)	12.8 (2.7)	13.2 (2.9)	13.1 (2.5)	13.1 (2.9)	NS
ETP (nM/min)	1583 (307)	1237 (384)	1189 (325)	1150 (372)	788 (322)	p = <0.0001
Peak height (nM)	229.3 (69.7)	187.6 (71.1)	184.8 (67.1)	187.1 (68.2)	147.8 (67.1)	p = <0.0001
ttPeak (min)	16.3 (2.8)	16.6 (3.3)	17.0 (3.4)	16.6 (2.8)	16.1 (3.0)	NS
Start tail (min)	36.4 (4.4)	36.1 (4.7)	36.3 (4.8)	35.5 (3.9)	34.1 (3.9)	p = <0.0001

Data are mean (n = 20, in triplicate) and standard deviation.

Key: ETP – endogenous thrombin potential; TM – thrombomodulin.

Table S3. Effect of APC concentrations on thrombin generation in healthy volunteers.

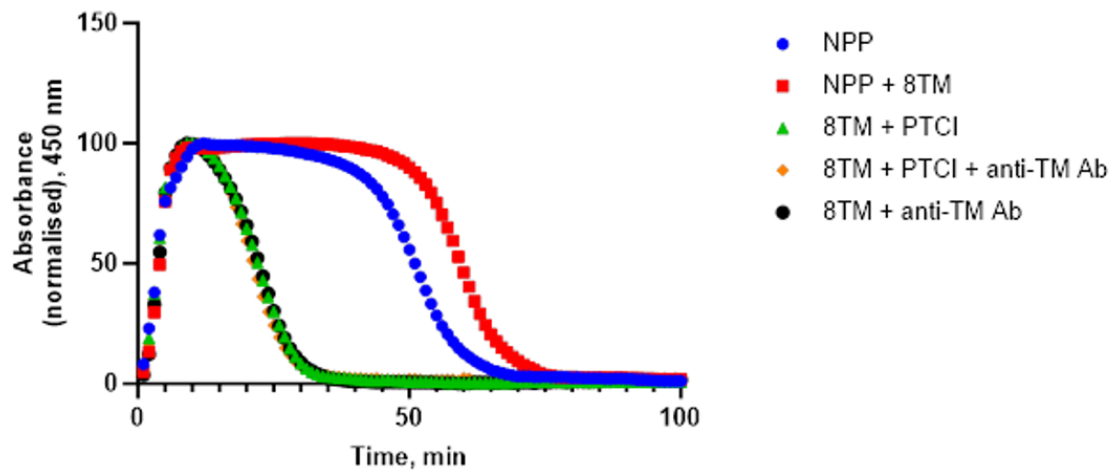
	Concentration of added activated protein C (pM)					One way ANOVA p value
	0 APC	50 APC	100 APC	200 APC	400 APC	
Lagtime (min)	12.95 (2.5)	14.2 (2.2)	13.59 (2.9)	14.13 (4.8)	13.38 (2.8)	NS
ETP (nM/min)	1583 (307)	1931 (939.5)	1658 (499.2)	2096 (542.5)	1748 (597.6)	NS
Peak height (nM)	229 (69.7)	254 (114.5)	224 (103.7)	300 (129.0)	240 (120.6)	0.005
ttPeak (min)	16.3 (2.8)	18.6 (3.0)	17.9 (3.7)	18.3 (6.0)	17.7 (3.7)	<0.0001
Start tail (min)	36.4 (4.4)	54.4 (5.1)	52.8 (6.2)	50.9 (6.0)	53.3 (5.9)	<0.0001

Data are mean (n = 20) and standard deviation.

Key: APC – activated protein C; ETP – endogenous thrombin potential.

Supplementary Figure 1.

Effects of PTCl and anti-thrombomodulin antibody on clot lysis.

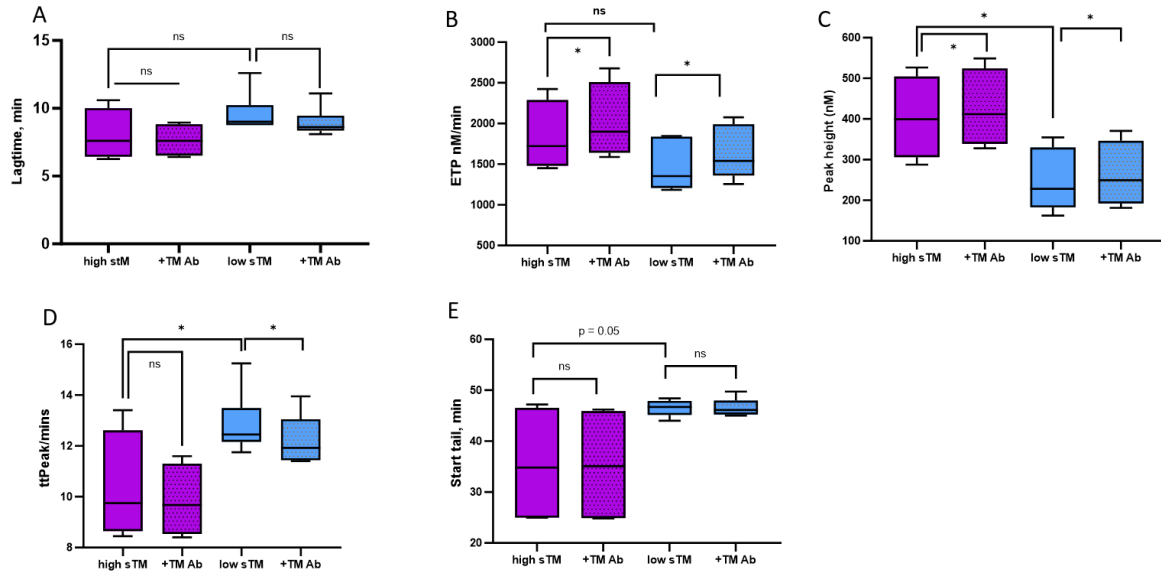


Data show curves of mean absorbance, detailing the effects of: 8ng/mL sTM, 50 ng/mL PTCl, 1 mcg/mL anti-TM antibody using normal pooled plasma on clot lysis. (Curves show the amalgamated mean data from each experimental condition, n = 3). Here the figure shows data only relating to an experimental condition using added thrombomodulin at 8ng/mL concentration. Similar effects across the 4 – 16 ng/mL sTM range were seen (data not shown).

Key. PTCl – potato tuber carboxypeptidase inhibitor; sTM – soluble thrombomodulin

Supplementary Figure 2.

Thrombin generation according to high sTM and low sTM trauma groups, with anti-TM antibody.



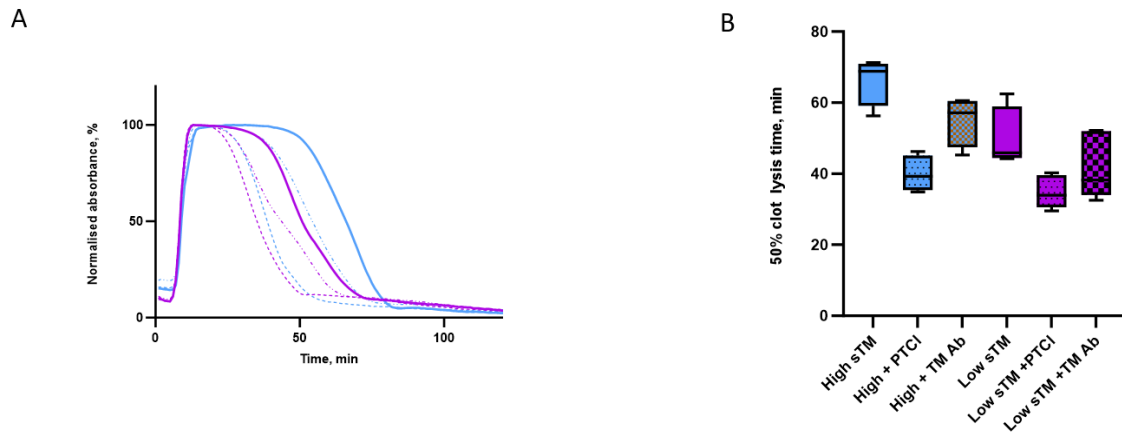
Thrombin generation parameters, comparing trauma patients with high sTM (purple) and low sTM (blue) levels, with and without anti-TM antibody, 1mcg/mL.

A: lagtime; B: Endogenous thrombin potential (ETP); C: Peak height; D: time to peak height; E: time to the start tail.

Key: ETP – endogenous thrombin potential; TM – thrombomodulin.

Supplementary Figure 3.

Clot lysis according to high sTM and low sTM trauma groups, with added PTCl or anti-TM antibody.



A. Data show amalgamated mean, normalised clot lysis curves for the trauma patients with the highest ($n = 4$) and lowest ($n = 5$) circulating sTM values. B. Data show median (IQR) 50% clot lysis times for those with the highest and lowest sTM values. Within groups, there was no significant differences between each group. The high sTM group had, on average, significantly longer times to 50% clot lysis ($p = 0.03$).

Key: purple lines – low sTM, blue lines – high sTM. Solid line: plasma alone, dotted line: plasma + 50 ng/mL PTCl, dot-dashed line: plasma + 1mcg/mL anti EGF5/6 TM antibody.