Clotting and Fibrinolysis: an integrated test system

Nijmegen Hemostasis Assay

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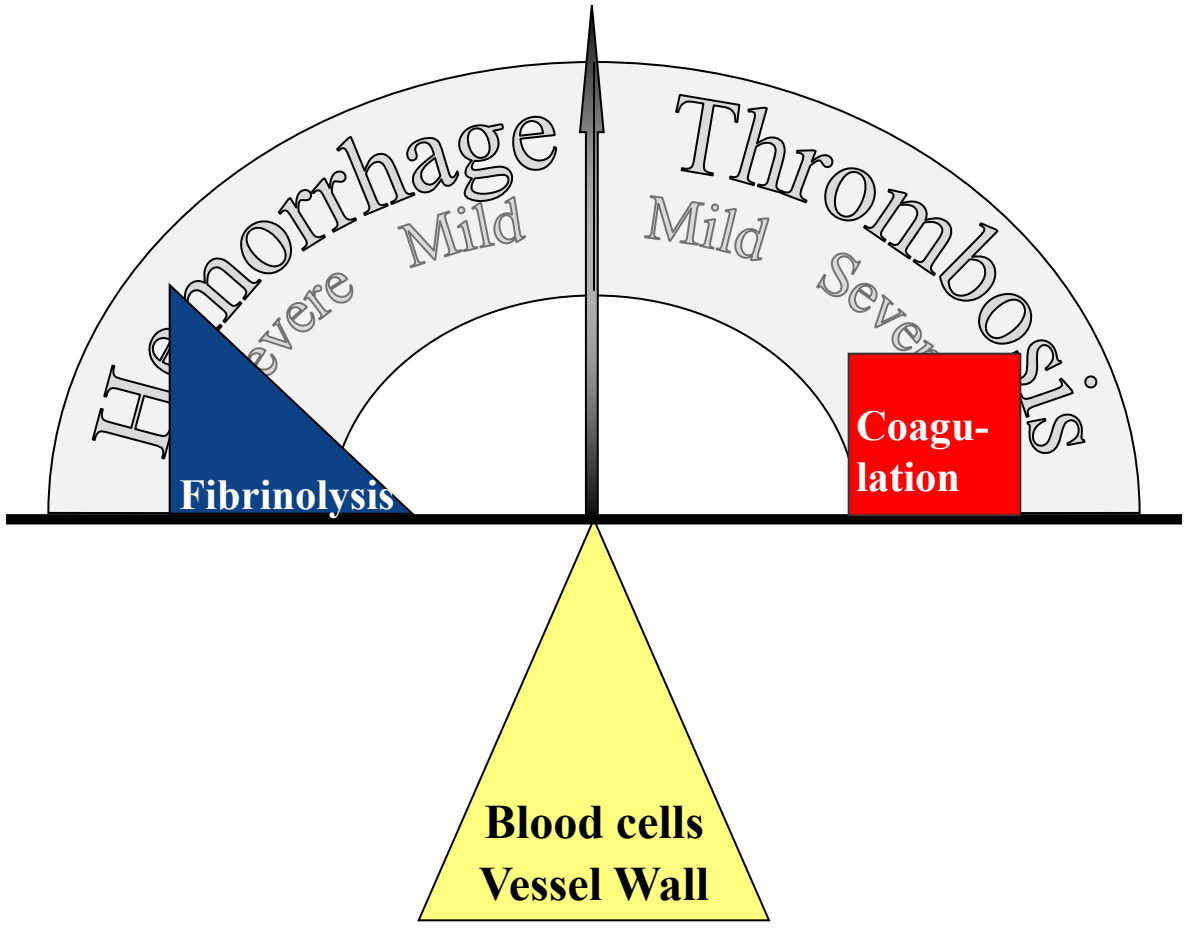
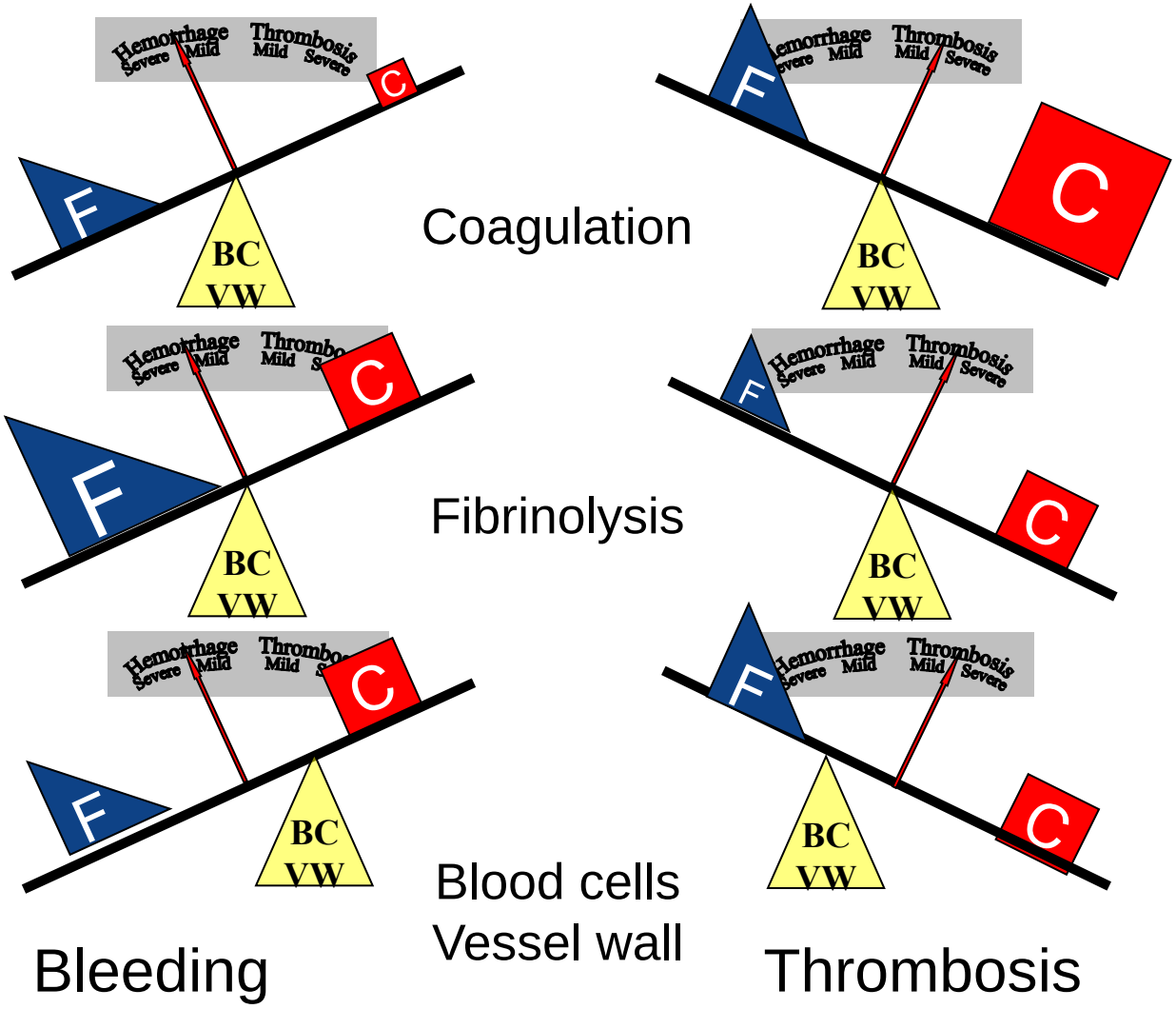
THOR

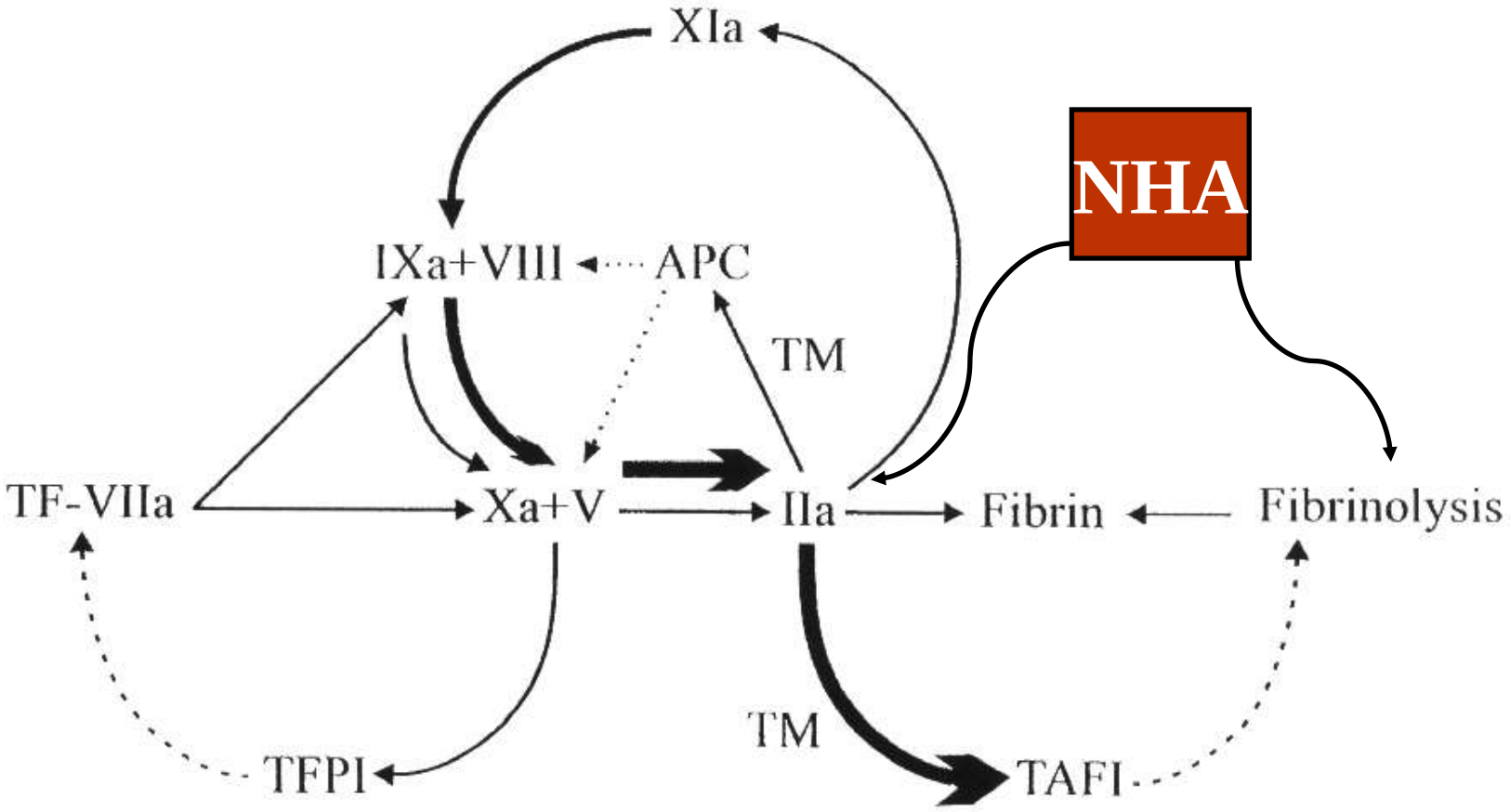
NHA: A tool to study the hemostatic balance



Deregulated hemostatic balance



NHA: Simultaneous detection of thrombin and plasmin



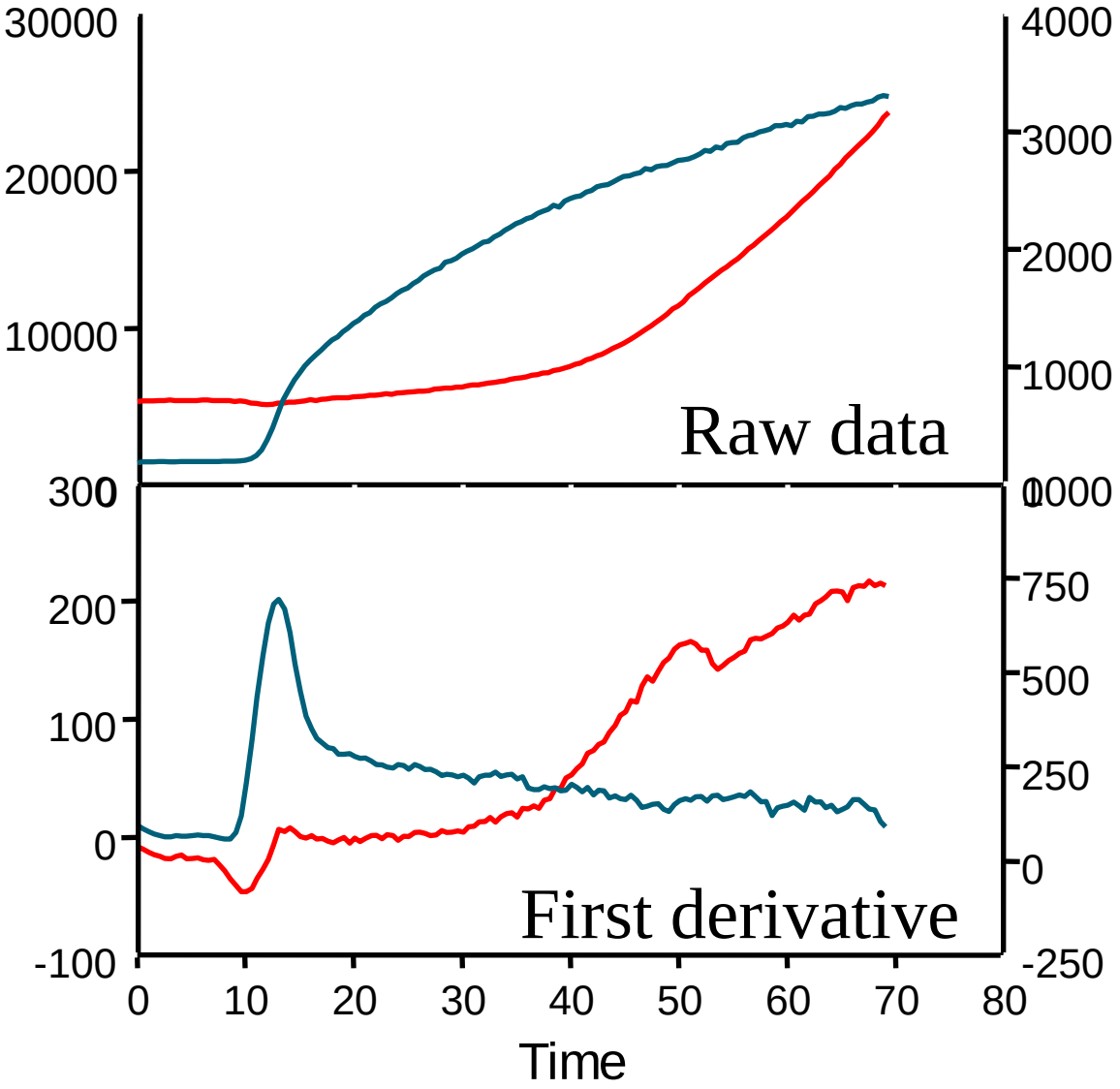
NHA specifications:

- Simultaneous detection of thrombin and plasmin generation in only 80 μL plasma

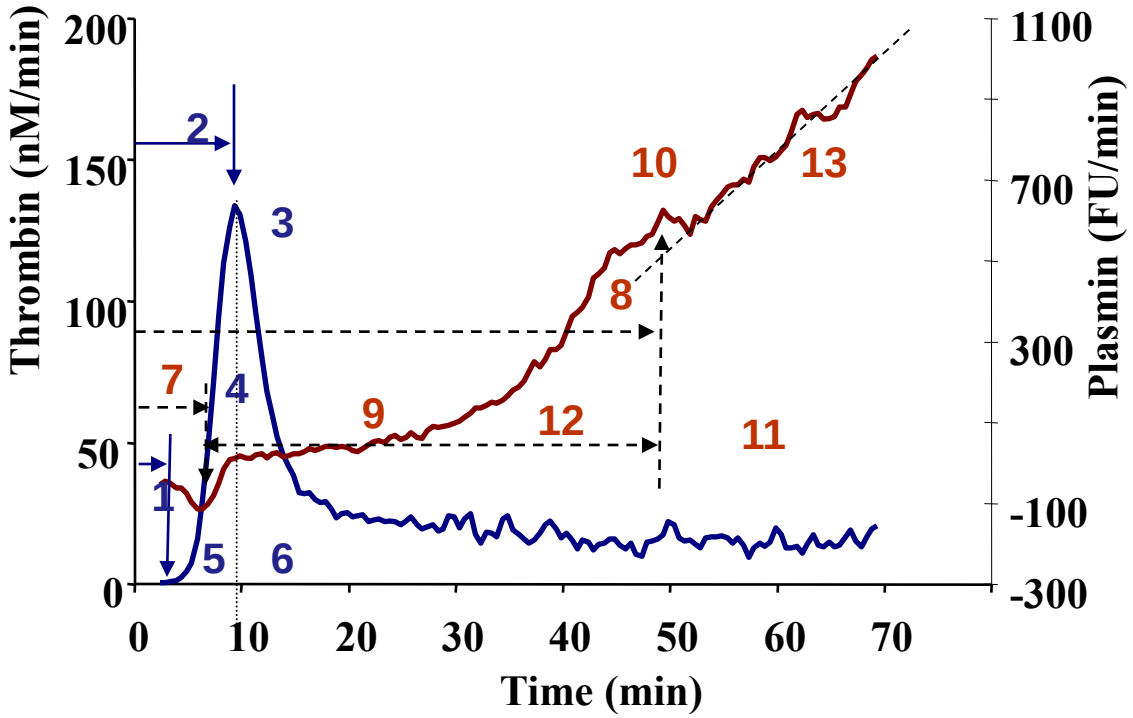
- Thrombin generation
  - Tissue Factor initiated
  - Phospholipid dependent

- Plasmin generation
  - t-PA initiated
  - Fibrin dependent

Principle measurement NHA



Characteristics and Parameters NHA

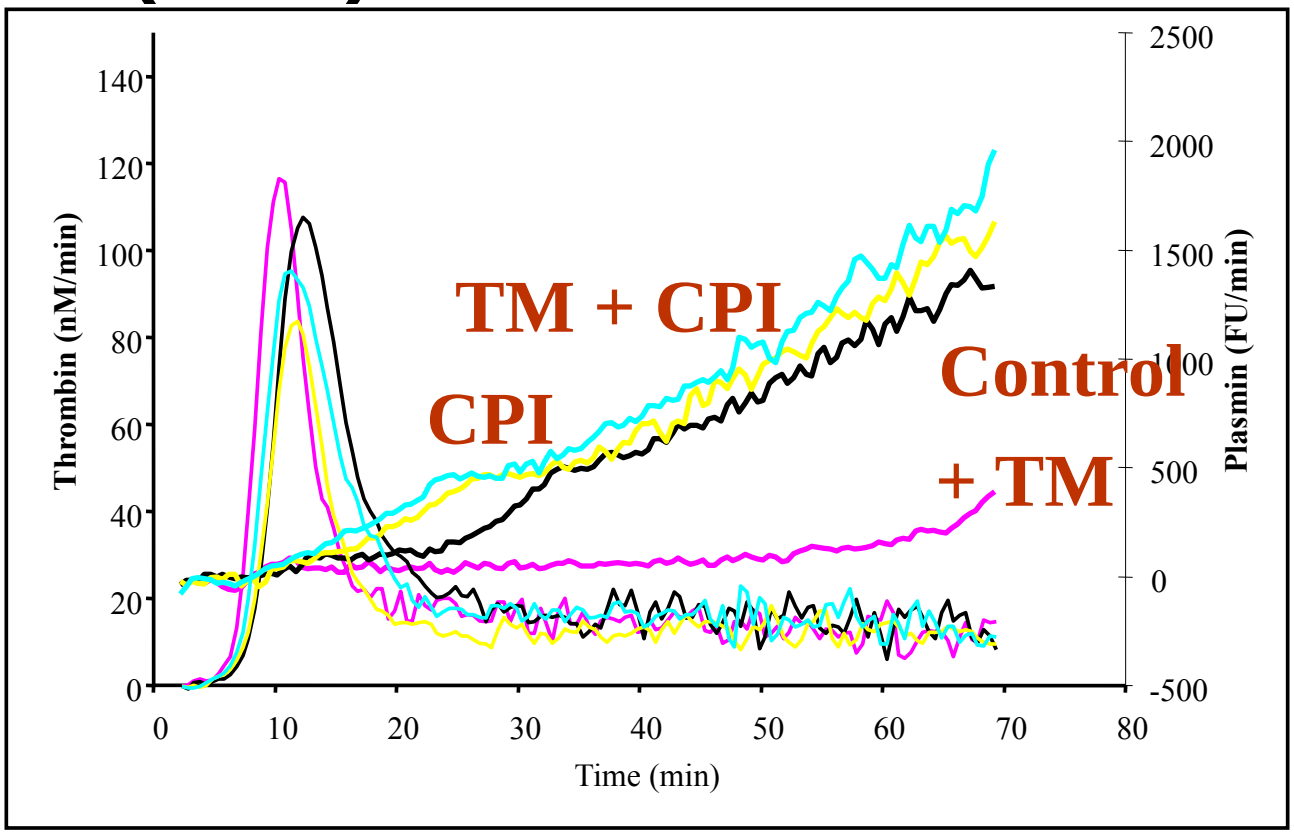


1: Lag Time Thrombin Generation
2: Time To Thrombin Peak
3: Thrombin Peak Height
4: Thrombin Potential (ETP)
5: ETP, 'procoagulant'
6: ETP, 'anticoagulant'
7: Plasmin Droptime
8: Plasmin Peaktime
9: Fibrin Lysis Time
10: Plasmin Peak Height
11: (PPeakHeight-PDropHeight)/FLT
12: Plasmin Potential, accel-phase
13: Plasmin Potential, PPT+10min

In vitro validation NHA (normal pooled plasma)

- Titration additives assay
  - TF, cephalin, t-PA
- Analysis several inhibitors
  - Hirudin, heparinoids, EACA, CTI, CPI
- Reproducibility
  - Intra assay variation
- Production of normal values using plasma of 50 healthy individuals

NHA and Thrombin Activatable Fibrinolysis Inhibitor(TAFI)

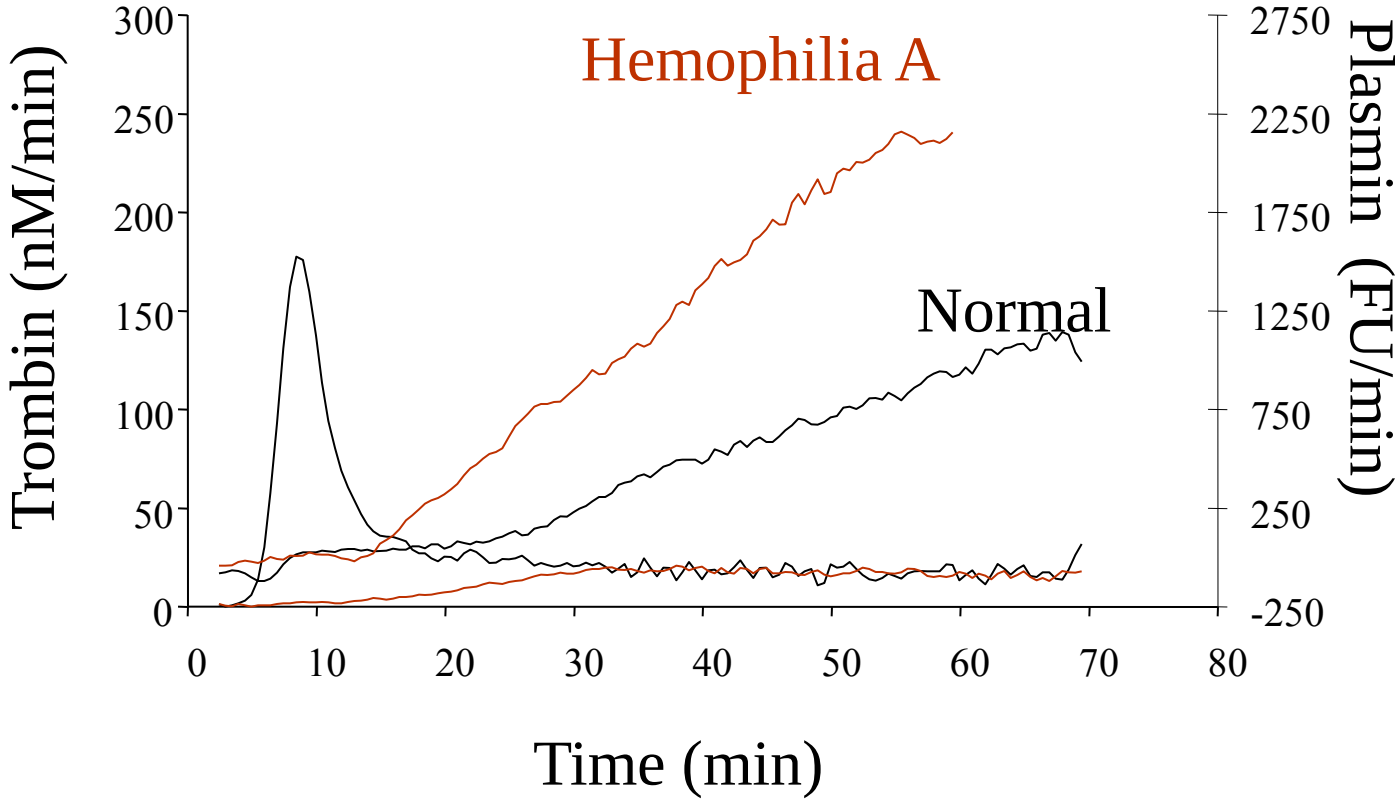


TM enhances TAFI activity, e.g. inhibition of plasmin generation. Carboxy peptidase inhibitor blocks TAFI act.

NHA validation in patients plasma

- Tested in “thrombophilic” patient plasma
  - Sensitive to PC, PS, AT, Factor V Leiden and prothrombin mutation
  - Sensitive to PAI-1 excess
- Tested in “hemophilic” patient plasma
  - Discriminative between initiation and propagation abnormalities in coagulation
  - Sensitive to fibrinogen deficiencies
  - Sensitive to PAI-1 and α_2-antiplasmin deficiencies

Severe Hemophilia A

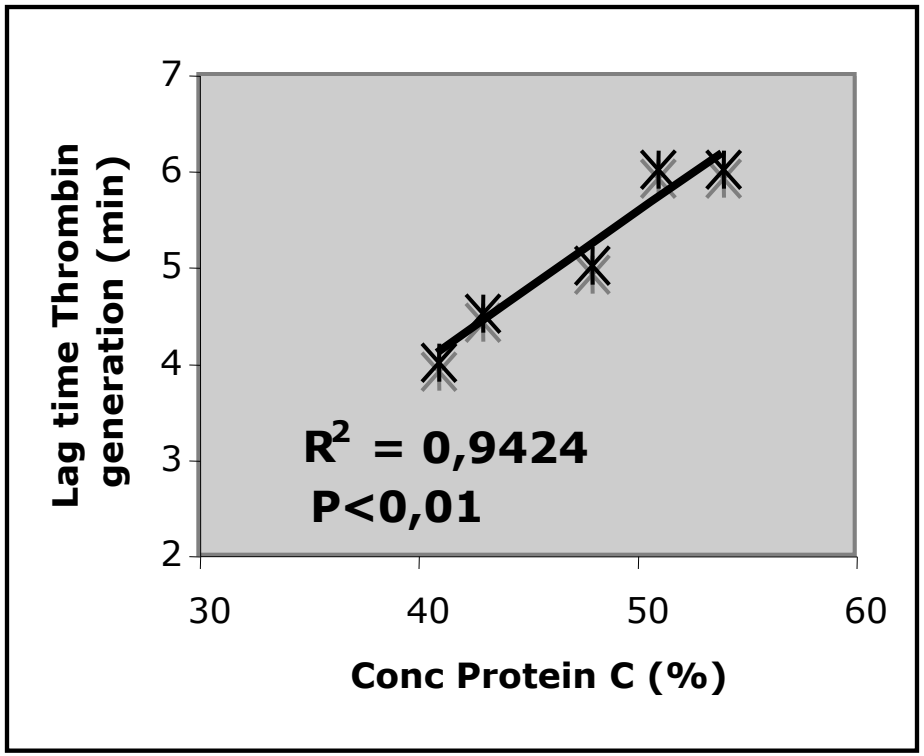


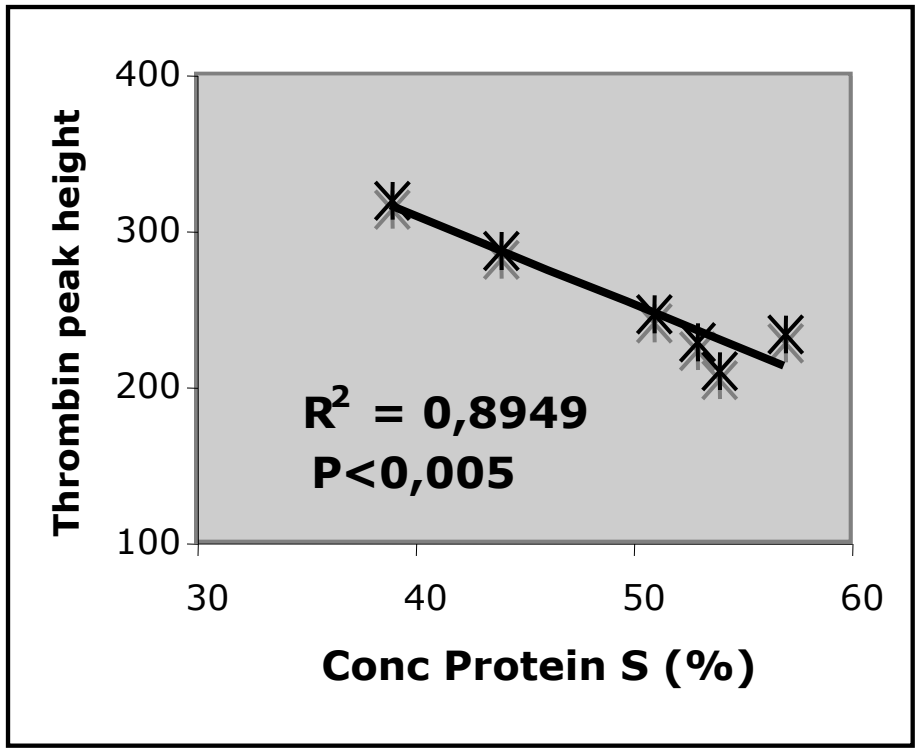
Diminished thrombin generation, no TAFI activation, increased fibrinolysis

NHA and Thrombophilia

	controls	FII G20210A	FV Leiden	PS def	PC Def
Genotype/Range		+/-	+/-	39-57%	41-54%
Numbers	45	6	6	6	5
Lag Time IIa Generation (min)	4.5 ± 0.7	5.1 ± 1.8	4.4 ± 0.7	4.4 ± 0.5	5.1 ± 0.9
Time To thrombin Peak (min)	9.0 ± 1.1	8.8 ± 2.3	*7.8 ± 0.8*	8.3 ± 1.0	8.9 ± 1.4
Max IIa Peak Height (nM/min)	234 ± 41	*340 ± 88*	260 ± 40	253 ± 41	253 ± 43
Thrombin Potential (ETP)	2308±377	2640±604	2385±422	2280±286	2226±248
ÑProcoagulantÓETP	456 ± 82	*579 ± 106*	416 ± 59	460 ± 85	423 ± 84
ÑAnticoagulantÓETP	1852 ± 32	2061 ± 553	1969 ± 414	1820 ± 268	1803 ± 234
Fibrin Lysis Time	34 ± 8	28 ± 9	28 ± 7	29 ± 10	28 ± 13

Correlation NHA with Protein C and Protein S





PC: No correlation with ETP

PS: No correlation with Lagtime

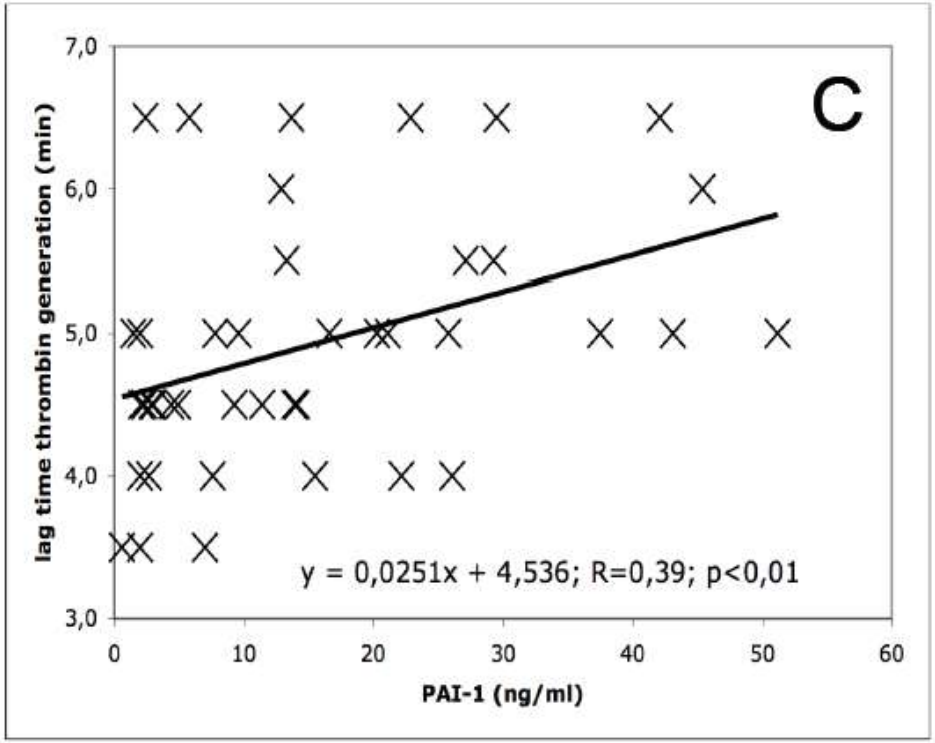
PS is a direct inhibitor of coagulation (anionic phospholipid binding protein)

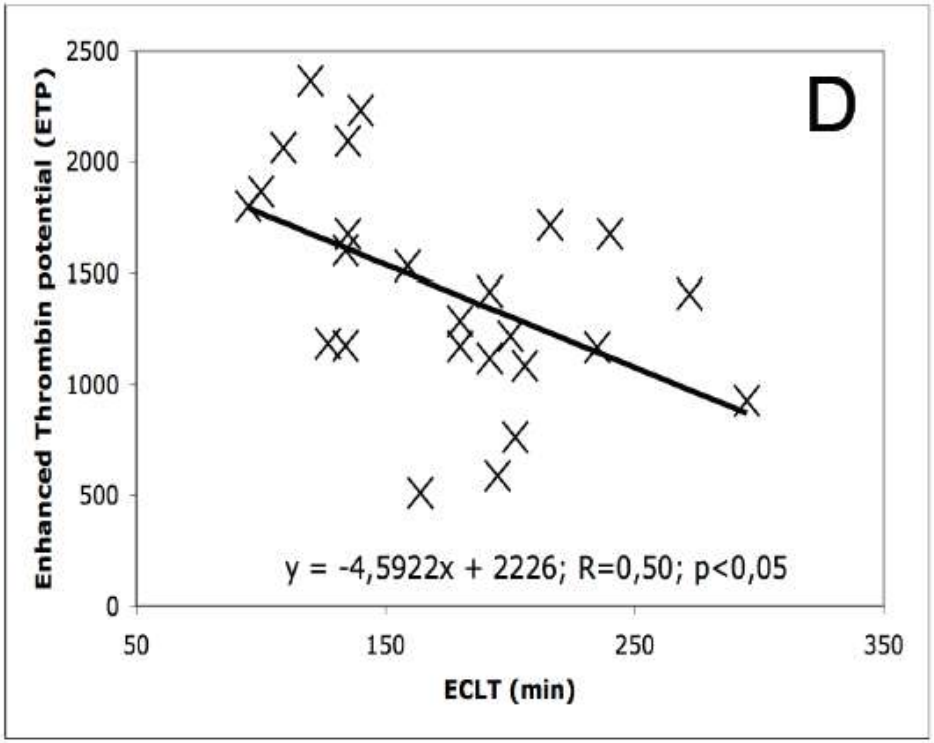
NHA validation in patients plasma

- Patients with a bleeding phenotype
  - Hemophilia A and B
  - Factor VII deficiency
  - Factor II, V, X, XI deficiency
  - Fibrinogen deficiency
  - Alpha-2-plasmin inhibitor and PAI-1 deficiency

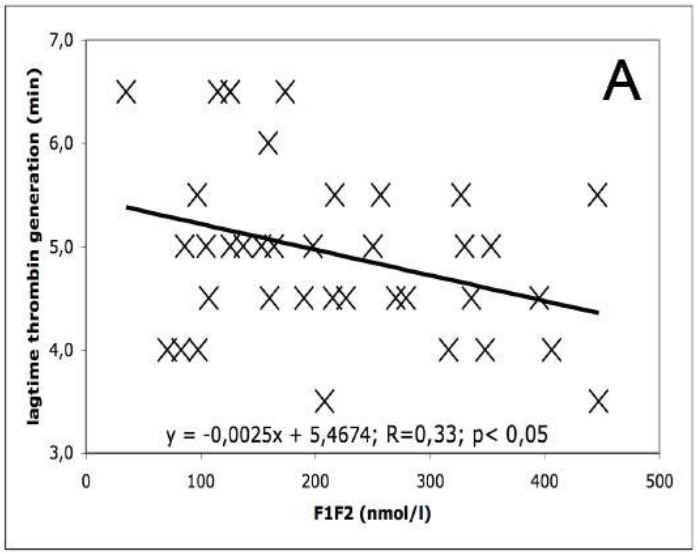
- Patients with a thrombophilic phenotype
  - Antithrombin, Protein C and S deficiency (heterozygous)
  - Factor V Leiden and Prothrombin Leiden
  - Sensitive to PAI-1 and α_2-antiplasmin deficiencies

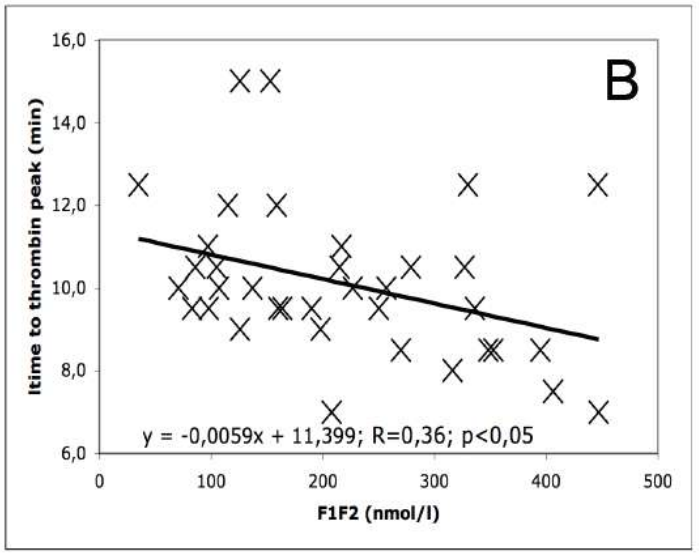
Relation fibrinolysis and coagulation cohort of patients with idiotypic bleeding disorders not related to coagulopathies

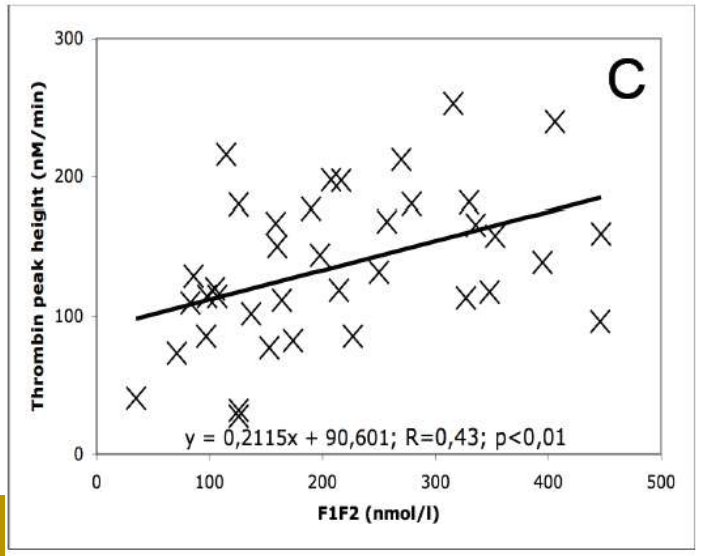


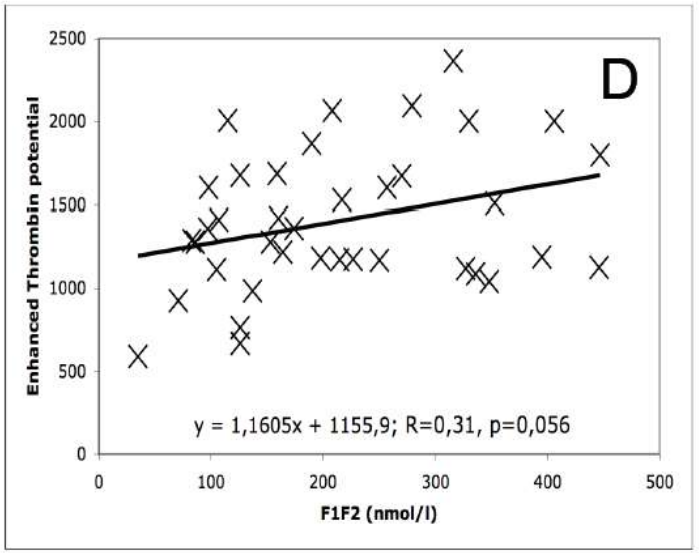


NHA and in vivo steady state level of coagulation cohort of patients with idiotypic bleeding disorders









Conclusions NHA

- New assay to detect aberrations in the hemostatic balance
- Applicable for screening
  - Hemophilia
  - Thrombophilia
  - Fibrinolysis
- Sensitive to interactions between coagulation and fibrinolysis (e.g. TAFI)
- Assay give new insights in hemostasis regulation