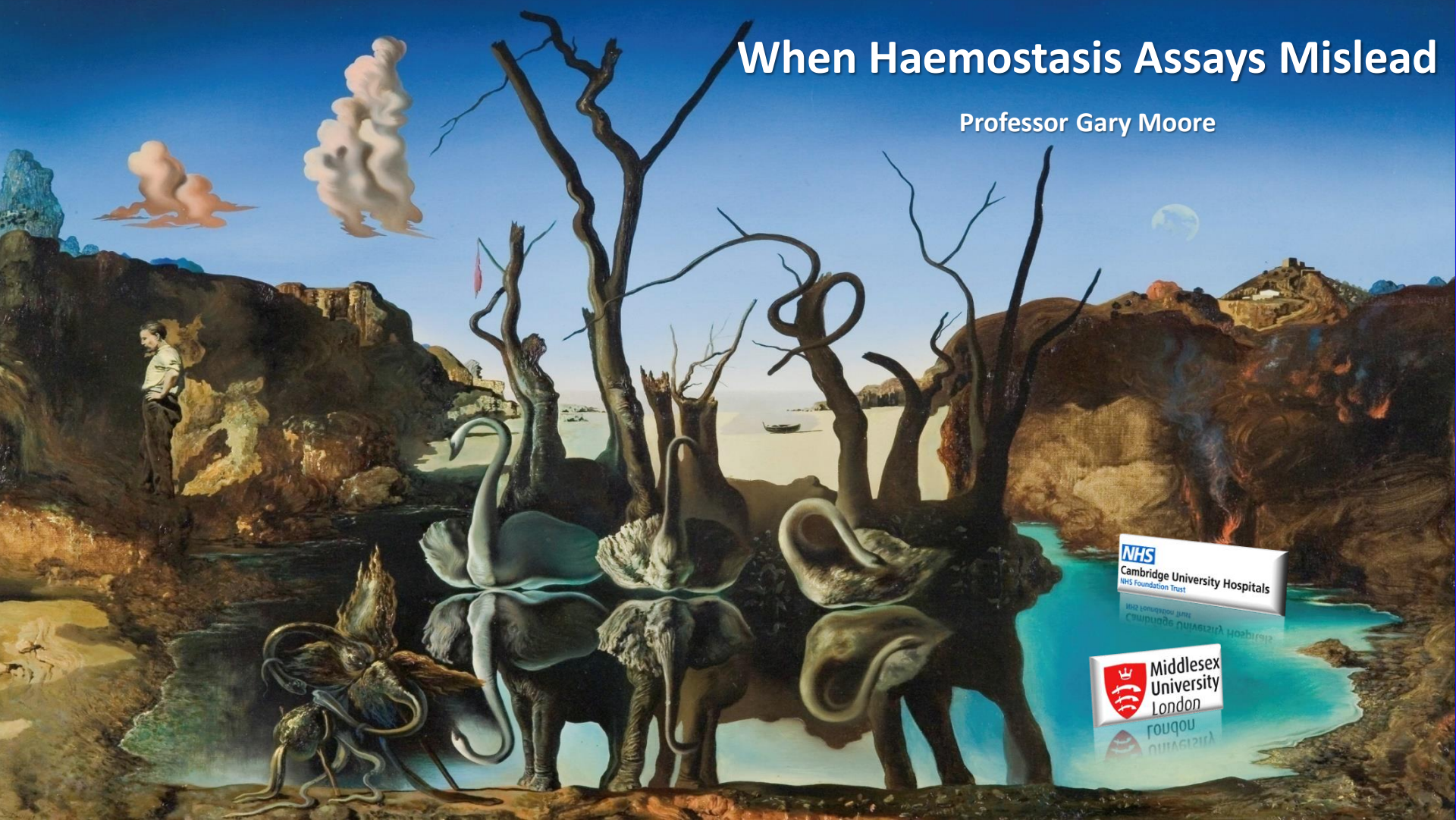
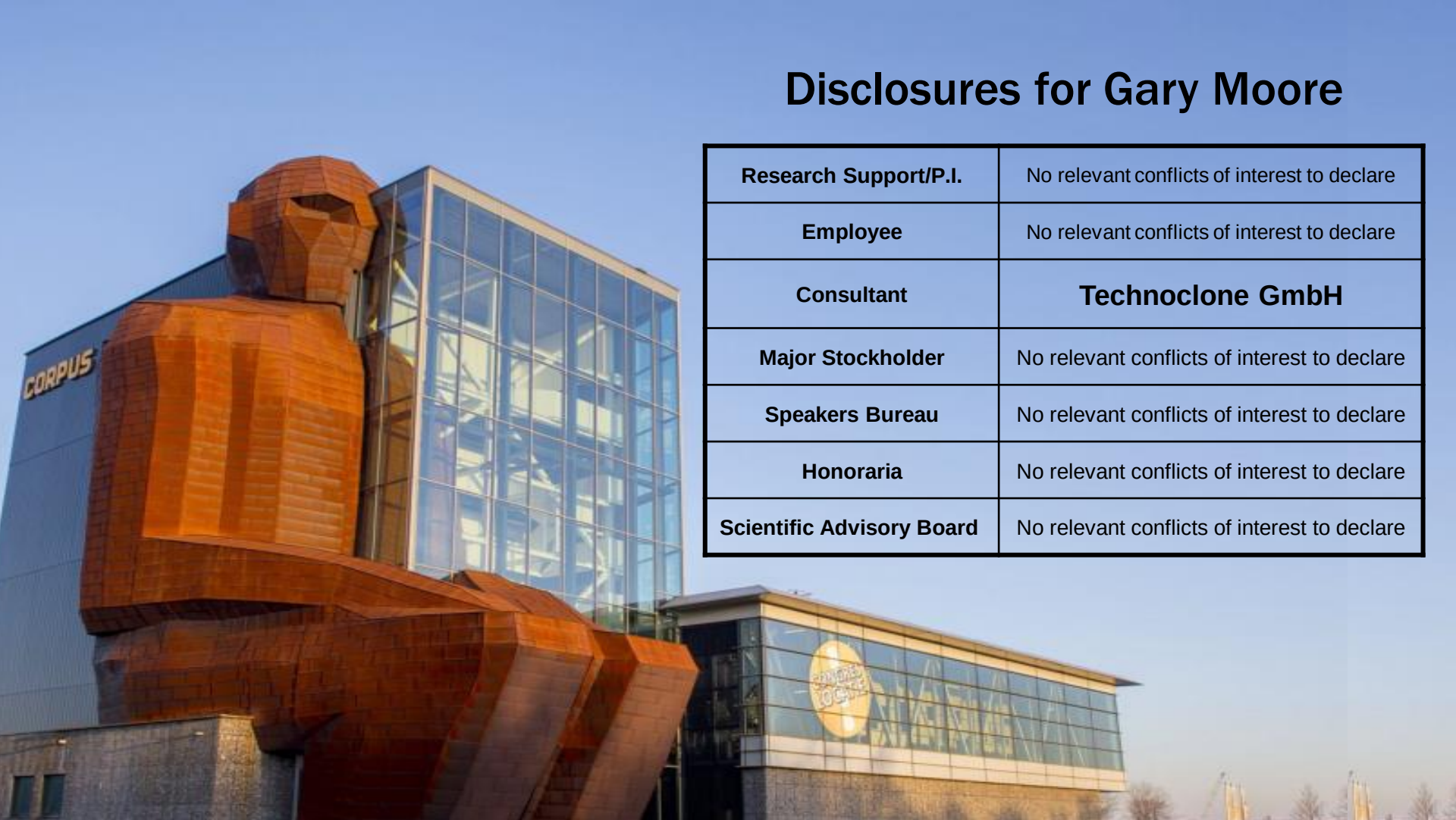
When Haemostasis Assays Mislead

Professor Gary Moore





Disclosures for Gary Moore

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Employee	No relevant conflicts of interest to declare
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Major Stockholder	No relevant conflicts of interest to declare
Speakers Bureau	No relevant conflicts of interest to declare
Honoraria	No relevant conflicts of interest to declare
Scientific Advisory Board	No relevant conflicts of interest to declare


Things aren't always what they seem


they may be something else


Hidden


Masked


Unusual


.... or unable to manifest


You might be using
the wrong tools


Screening tests

9 year old boy

Non-consanguineous parents

Mild bleeding tendency: easy bruising, occasional epistaxis, bled excessively after tonsillectomy
some paternal relatives, including father, had mild bleeding tendency

Lab 1			
Assay		**Result**	**RR**
PT	(s)	12	10 – 13
APTT	(s)	35	30 - 40
TT	(s)	9	9 - 11

Lab 2			
Assay		**Result**	**RR**
PT	(s)	**30**	12 – 15
PT 50:50 mix	(s)	14	12 - 15
APTT	(s)	37	32 – 42
TT	(s)	11	10 - 12
FVII:C	(iu/dL)	**7**	50 – 150

FVII Padua

Factor VII Deficiency: From Basics to Clinical Laboratory Diagnosis and Patient Management

Pierre-Olivier Sevenet, PharmD[1], Daniel A. Kaczor, BS, MT[2], and Francois Depasse, PharmD, MSc[1]

Clinical and Applied Thrombosis/Hemostasis
2017, Vol. 23(7) 703-710

DOI: 10.1177/1076029616670257
journals.sagepub.com/home/cat
SAGE

Table 2. Factor VII:C Activity of Different Homozygous FVII Defects According to Girolami et al.[27]

	FVII Padua (Arg304Gln)	FVII Nagoya (Arg304Trp)	FVII Tondabaya-shi or Shinjo (Arg79Gln)
Mean FVII:C (rabbit brain thromboplastin)	6%	5%	7%
Mean FVII:C (human recombinant thromboplastin)	34%	16%	48%
Mean FVII:C (ox brain thromboplastin)	101%	60%	109%
FVII:Ag	>100%	100%	93%

- Asymptomatic/mild bleeding tendency
- Type II defects with variable activity
- FVII:Ag normal or near normal

Factor VII Padua$_2$: Another Factor VII Abnormality With Defective Ox Brain Thromboplastin Activation and a Complex Hereditary Pattern

By A. Girolami, G. Cattarozzi, R. Dal Bo Zanon, G. Cella, and F. Toffanin

blood
Blood, Vol. 54, No. 1 (July), 1979

81 year old woman

Admitted for radiofrequency ablation of renal tumour in June 2017

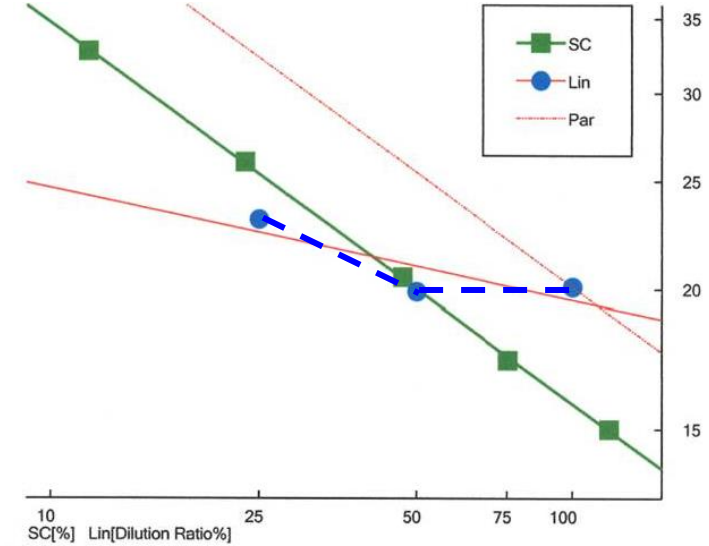
No bleeding symptoms or history of bleeding – apparently not anticoagulated

Coagulation screen - June 2017		
Assay	**Result**	**RR**
PT/INR	**2.3**	0.8 – 1.2
PT/INR 50:50 mix	**1.4**	0.8 – 1.2
APTT (ratio)	1.0	0.8 – 1.2

Coagulation screen – Sept. 2017		
Assay	**Result**	**Reference range**
PT/INR	**2.7**	0.8 – 1.2
APTT (ratio)	1.2	0.8 – 1.2
Fibrinogen (g/L)	3.1	1.7 – 4.0

Vitamin K epoxide <0.27 µg/L

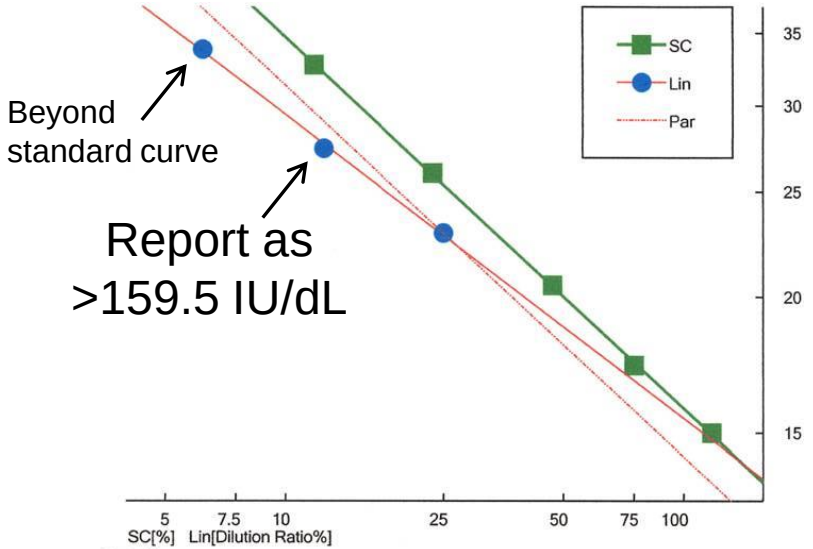
1-stage FVII assay with recombinant human thromboplastin



Detail
0000.0000.0000 Dilution Ratio was Changed.

MDA Point

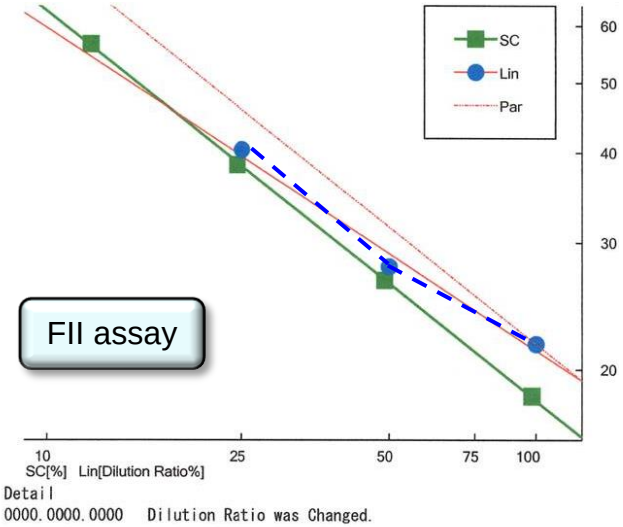
Replications	Dil. Ratio	sec	VII % %
1	1/1	20.1	49.4
1	1/2	19.9	101.8
1	1/4	23.1	131.5
		Mean	94.2



Detail
0000.0000.0000 Dilution Ratio was Changed.

MDA Point

Replications	Dil. Ratio	sec	VII % %
1	1/4	22.9	134.9
1	1/8	27.4	159.5
1	1/16	33.8	173.9
		Mean	156.1



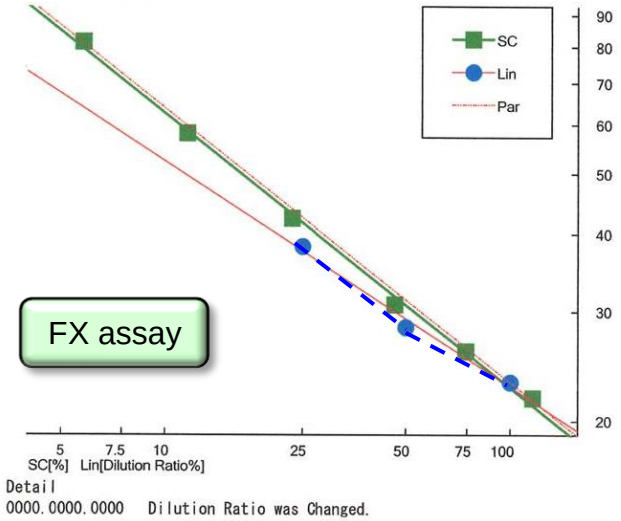
FII assay

Inhibition abolished & parallelism restored at 1/20

FII = 90.8 IU/dL

MDA Point

Replications	Dil. Ratio	sec	II % %
1	1/1	21.7	71.7
1	1/2	27.8	90.7
1	1/4	40.4	90.9
		Mean	84.4



FX assay

Inhibition abolished & parallelism restored at 1/20

FX = 121.6 IU/dL

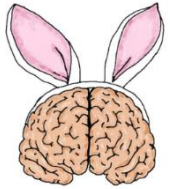
MDA Point

Replications	Dil. Ratio	sec	X % %
1	1/1	23.1	95.7
1	1/2	28.3	120.8
1	1/4	38.2	122.4
		Mean	113.0

APTT-based one-stage FIX assay was normal and without interference

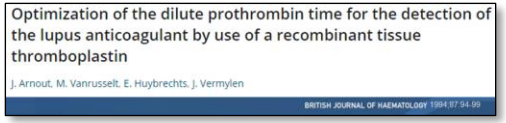
Lupus anticoagulant assays			
Assay	**Units**	**Result**	**RR**
dRVVT screen	(ratio)	**1.23**	0.85 – 1.17
dRVVT confirm	(ratio)	0.91	0.90 – 1.10
% correction	(%)	**26.0**	≥ 10
dRVVT screen 1:1 mix	(ratio)	**1.37**	0.90 – 1.07
dRVVT confirm 1:1 mix	(ratio)	0.99	0.98 – 1.10
dAPTT screen	(ratio)	**1.46**	0.80 – 1.20
dAPTT confirm	(ratio)	0.99	0.82 – 1.18
% correction	(%)	**32.2**	≥ 10
dAPTT screen 1:1 mix	(ratio)	**1.38**	0.86 – 1.10
dAPTT confirm 1:1 mix	(ratio)	0.88	0.88 – 1.12

However, these are not extrinsic pathway-based assays so how do we know it was the LA affecting the PT?



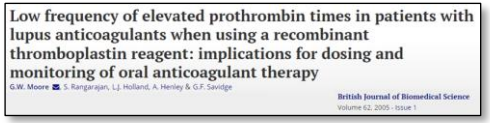
Rabbit brain thromboplastin

INR 1.0


Optimization of the dilute prothrombin time for the detection of the lupus anticoagulant by use of a recombinant tissue thromboplastin

J. Arnout, M. Vanrusselt, E. Huybrechts, J. Vermylen

BRITISH JOURNAL OF HAEMATOLOGY 1994;87:94-99

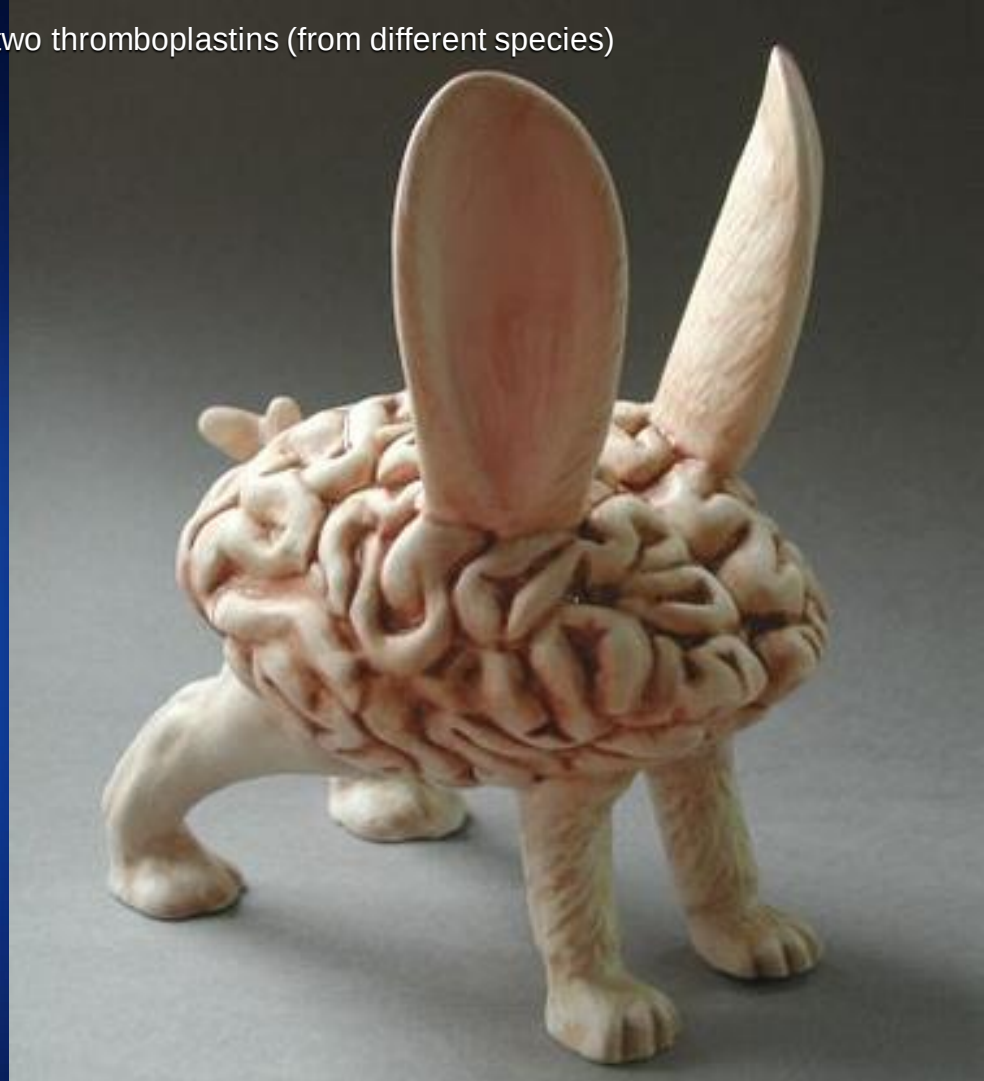

Low frequency of elevated prothrombin times in patients with lupus anticoagulants when using a recombinant thromboplastin reagent: implications for dosing and monitoring of oral anticoagulant therapy

G.W. Moore, S. Rangarajan, L.J. Holland, A. Henley & G.F. Savidge

British Journal of Biomedical Science

Volume 62, 2005 - Issue 1

Every haemostasis laboratory needs two thromboplastins (from different species)



56 year old female

Non-consanguineous parents

Asymptomatic

Lab 1			
Assay		**Result**	**RR**
PT	(s)	**22**	10 – 13
PT 50:50 mix	(s)	12	10 - 13
APTT	(s)	38	30 - 40
TT	(s)	9	9 - 11
FVII:C	(IU/dL)	120	50 - 150
LA testing by dRVVT, APTT & dPT			Not detected
FX:C	(IU/dL)	**25**	50 - 150

Lab 2			
Assay		**Result**	**RR**
PT	(s)	**21**	12 – 15
PT 50:50 mix	(s)	13	12 - 15
APTT	(s)	30	32 – 42
TT	(s)	11	10 - 12
FVII:C	(IU/dL)	117	50 - 150
FX:C	(IU/dL)	**27**	50 - 150

FX Padua

reduced activity with FX chromogenic assay & extrinsic assays

normal activity with Russell's viper venom & intrinsic assays

normal FX:Ag

Haemophilia

Persistent validity of a classification of congenital factor X defects based on clotting, chromogenic and immunological assays even in the molecular biology era

A. GIROLAMI, S. VETTORE, P. SCARPARO and A. M. LOMBARDI

Haemophilia (2011), **17**, 17–20

Type I	Concordant reduction in activity and antigen	Stuart-like
Type II	Inert protein but measurable antigen	Prower-like
Type III	Dysreactive protein and measurable (higher) antigen	
	1. Defects in all activity assays except RVV	Friuli-like
	2. Defects only or predominantly in extrinsic-Xase	Padua-like
	3. Defects only or predominantly in intrinsic-Xase	Melbourne-like
	4. Defects with higher activity levels in chromogenic assays	
Type IV	Defect associated with other clotting factor deficiencies	i.e. FVII & chromosome 13 abnormalities

8 day old boy given vitamin K at birth - severe bleeding after circumcision

Lab 3			
Assay		**Result**	**RR**
PT	(s)	**75**	30 – 40
PT 50:50 mix	(s)	**45**	30 – 40
APTT	(s)	**130**	32 – 42
APTT 50:50 mix	(s)	40	32 – 42
TT	(s)	11	10 - 12

FII:C	(iu/dL)	75	50 – 150
FV:C	(iu/dL)	105	50 – 150
FVII:C	(iu/dL)	55	50 – 150
FX:C	(iu/dL)	62	50 – 150

Lab 1			
Assay		**Result**	**RR**
PT	(s)	11	10 – 13
APTT	(s)	**120**	30 - 40
APTT 50:50 mix	(s)	38	30 - 40
TT	(s)	9	9 - 11

FVIII:C	(iu/dL)	145	50 – 150
FIX:C	(iu/dL)	**<1**	50 – 150
FXI:C	(iu/dL)	80	58 – 120
FXII:C	(u/dL)	89	50 – 150

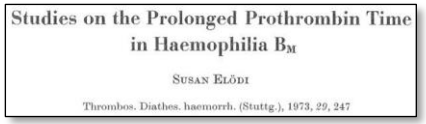
Haemophilia B_M

Markedly prolonged PT with bovine (ox)-brain thromboplastin

May have mildly prolonged PT with other thromboplastins

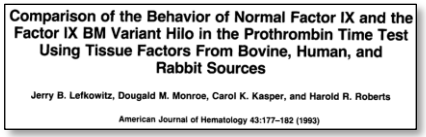
Can be accompanied by genuine, mild FVII deficiency

FIX Deventer
FIX Milano
FIX Novara
FIX Bergamo
FIX Hilo
FIX Lake Elsinore


Studies on the Prolonged Prothrombin Time in Haemophilia B_M

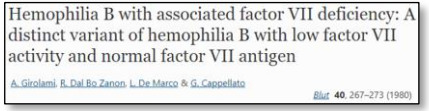
Susan Elödi

Thrombos. Diathes. haemorrh. (Stuttg.), 1973, *29*, 247


Comparison of the Behavior of Normal Factor IX and the Factor IX BM Variant Hilo in the Prothrombin Time Test Using Tissue Factors From Bovine, Human, and Rabbit Sources

Jerry B. Lefkowitz, Dougald M. Monroe, Carol K. Kasper, and Harold R. Roberts

American Journal of Hematology 43:177–182 (1993)

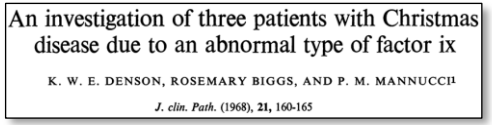

Hemophilia B with associated factor VII deficiency: A distinct variant of hemophilia B with low factor VII activity and normal factor VII antigen

A. Girolami, R. Dal Bo Zanon, L. De Marco & G. Cappellato

Blut **40**, 267–273 (1980)

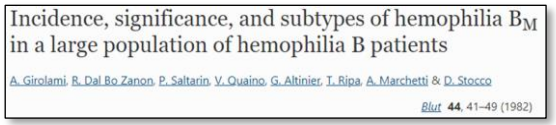
FIX:Ag normal – dysfunctional molecule

Inhibitory effect of abnormal FIX in bovine brain PT of TF-dependent FX activation


An investigation of three patients with Christmas disease due to an abnormal type of factor ix

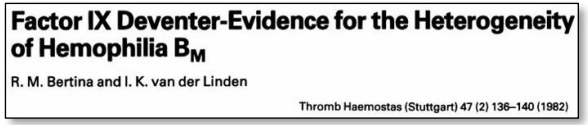
K. W. E. DENSON, ROSEMARY BIGGS, AND P. M. MANNUCCI

J. clin. Path. (1968), **21**, 160-165


Incidence, significance, and subtypes of hemophilia B_M in a large population of hemophilia B patients

A. Girolami, R. Dal Bo Zanon, P. Saltarin, V. Quaino, G. Altinier, T. Ripa, A. Marchetti & D. Stocco

Blut **44**, 41–49 (1982)


Factor IX Deventer-Evidence for the Heterogeneity of Hemophilia B_M

R. M. Bertina and I. K. van der Linden

Thromb Haemostas (Stuttgart) 47 (2) 136–140 (1982)

23 year old male

Right leg occlusive femoral-popliteal DVT after mild muscular stretching (3 days previously)

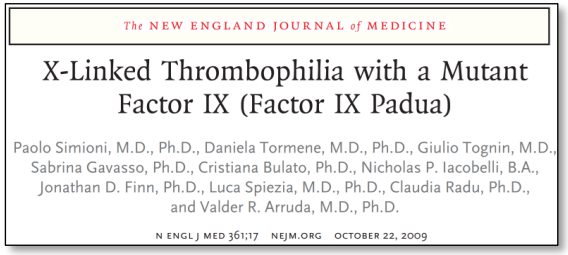
No FH of thrombosis

Assay		Result	RR
PT	(s)	11	10 - 13
APTT	(s)	**26**	30 - 40
TT	(s)	10	9 - 11
Normal antithrombin, protein C, free protein S & APC-R			

Assay		Result	RR
PT	(s)	12	10 - 13
APTT	(s)	**25**	30 - 40
Fibrinogen	(g/L)	3.2	2.0 – 4.0
FVIII:C	(iu/dL)	100	50 - 150

Causes of shortened APTT:

difficult venepuncture leading to activated sample	**spurious result**
analytical error	**spurious result**
overfilled sample	**spurious result**
natural statistical outlier	**genuine result**
elevated FVIII (innate, acquired)	**genuine result**
elevated fibrinogen	**genuine result**



The NEW ENGLAND JOURNAL of MEDICINE

X-Linked Thrombophilia with a Mutant Factor IX (Factor IX Padua)

Paolo Simioni, M.D., Ph.D., Daniela Tormene, M.D., Ph.D., Giulio Tognin, M.D., Sabrina Gavasso, Ph.D., Cristiana Bulato, Ph.D., Nicholas P. Iacobelli, B.A., Jonathan D. Finn, Ph.D., Luca Spiezia, M.D., Ph.D., Claudia Radu, Ph.D., and Valder R. Arruda, M.D., Ph.D.

N ENGL J MED 361;17 NEJM.ORG OCTOBER 22, 2009

Gain of function mutation

Substitution of leucine for arginine at position 338 (FIX-R338L)

X-linked thrombophilia

Table 1. Clinical Characteristics and Laboratory Data from the Family Members.*

Subject	Sex	Age (yr)	Activated Partial-Thromboplastin Time (sec)†	Factor IX Antigen (% of normal level)	Factor IX Activity (% of normal level)	Factor IX Activity-to-Antigen Ratio
II-1, proband	M	23	25.7	92	776	8.4
I-1 , father	M	53	35.2	105	127	1.2
I-2 , mother	F	46	28.2	94	337	3.5
II-2 , brother	M	21	33.4	116	123	1.0
II-3 , brother	M	11	29.1	64	551	8.6

† The normal range for activated partial-thromboplastin time is 30 to 40 seconds.

During warfarin therapy:

INR	3.4
FIX:C	160%
FIX:Ag	28%

Partial *F8* gene duplication (factor VIII Padua) associated with high factor VIII levels and familial thrombophilia

Paolo Simioni,[1] Stefano Cagnin,[2-4] Francesca Sartorello,[1] Gabriele Sales,[2] Luca Pagani,[2,5] Cristiana Bulato,[1] Sabrina Gavasso,[1] Francesca Nuzzo,[6] Francesco Chemello,[2] Claudia M. Radu,[1] Daniela Tormene,[1] Luca Spiezia,[1] Tilman M. Hackeng,[6] Elena Campello,[1] and Elisabetta Castoldi[6]

blood® 29 APRIL 2021 | VOLUME 137, NUMBER 17 2383

First reported thrombophilic defect in *F8*

Described in two Italian families

23.4.kb tandem duplication of proximal portion of *F8*

Associated with elevated FVIII & VTE

Affects males & females

Table 1. Clinical characteristics and laboratory data of the family members

Participant	Sex	Age, y	Thrombotic events	Age at first VTE, y	PT/INR	aPTT, s*	FVIII:C, %†	FVIII:Ag, %‡	VWF:Ag, %§	Additional thrombophilic defects
Family A										
II-3, proband	F	53‖	DVT and PE	31	2.88¶	26.5¶	422	432	165	No
I-4	F	77	DVT and PE	49	1.00	21.6	269	296	144	No
II-6	M	45	SVT and DVT	43	0.98	21.1	416	372	60	No
III-1	M	26	No	—	1.11	29.3	132	152	144	No
III-2	M	22	No	—	1.1	27.2	323	368	112	No
Family B										
II-1, proband	M	41	DVT and PE	21	3.1¶	40¶	264	273	78	Heterozygous FV Leiden
I-2	F	66	DVT	24	0.93	22.2	508	457	68	Heterozygous FV Leiden
III-1	F	19	No	—	1.07	26.2	295	270	113	Heterozygous FV Leiden

*Normal range, 26 to 34 seconds.

†Normal range, 58% to 162%.

‡Normal range, 64% to 156%.

§Normal range, 52% to 178%.

‖Age at death.

¶VKA treatment.

53 year old male

4 months post cardiac surgery

No bleeding symptoms

Assay		Result	RR
PT	(s)	12	10 – 13
APTT	(s)	36	30 - 40
TT	(s)	**>240**	9 - 11
Reptilase time	(s)	13	12 – 16
Fibrinogen	(g/L)	4.0	2.0 – 4.0

NOT

UFH	APTT normal
LMWH	would not elevate TT that high
Dysfibrinogenemia	Clauss fibrinogen too high
Elevated D-dimers	would affect reptilase time to some extent
Paraprotein	would likely affect reptilase time
Dabigatran	would affect APTT at that level

CAUSE

Antibody to bovine thrombin

Normal thrombin time with human thrombin reagent

49 year old female

Post-op coagulation screen

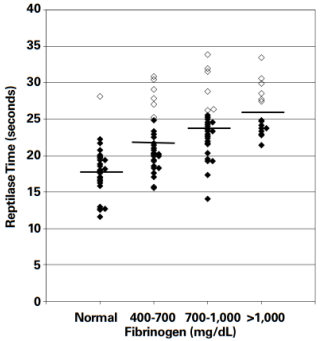
No other information

Assay		Result	RR
PT	(s)	11	10 – 13
APTT	(s)	**50**	30 - 40
Fibrinogen	(g/L)	**8.3**	2.0 – 4.0
TT	(s)	10	9 - 11
RT	(s)	**24**	12 - 16
FVIII:C	(IU/dL)	**208**	50 - 150
CRP	(mg/L)	**120**	0 - 3

Elevated Fibrinogen in an Acute Phase Reaction Prolongs the Reptilase Time but Typically Not the Thrombin Time

Elizabeth M. Van Cott, MD,[1] Eve Y. Smith,[1] and Dennis K. Galanakis, MD[2]

Am J Clin Pathol 2002;118:263-268



? acute phase reaction generates fibrinogen with increased sialic acid &/or phosphorous content

Effect abolished by adding 3 mmol/L $CaCl_2$ or 2.5% albumin – not abolished if hereditary dysfibrinogenemia

TT & RT used to screen for dysfibrinogenemia – prolonged

Fibrinogen Oslo I associated with thrombosis

Fibrinogen Valhalla associated with bleeding

Both give shortened TT


Factor assays

International Journal of Laboratory Hematology

The Official journal of the International Society for Laboratory Hematology

The responsiveness of different APTT reagents to mild factor VIII, IX and XI deficiencies

A. BOWYER*,†, S. KITCHEN*, M. MAKRIS*,†

Int. Jnl. Lab. Hem. 2011, **33**, 154–158

International Journal of Laboratory Hematology

The Official journal of the International Society for Laboratory Hematology

Determination of APTT factor sensitivity – the misguiding guideline

A. S. LAWRIE*, S. KITCHEN†, M. EFTHYMIOU*, I. J. MACKIE*, S. J. MACHIN*

Int. Jnl. Lab. Hem. 2014, **35**, 652–657

Investigation of sensitivity for coagulation factor deficiency in APTT and PT: how to perform it?

Els N. Dumoulin, Lisse Fiers and Katrien M.J. Devreese*

Clin Chem Lab Med 2016; 54(5): e169–e172

International Journal of Laboratory Hematology

The Official journal of the International Society for Laboratory Hematology

***In vitro* sensitivity of different activated partial thromboplastin time reagents to mild clotting factor deficiencies**

P. TOULON*,†, Y. ELOIT†, M. SMAHI‡, C. SIGAUD†, D. JAMBOU†, F. FISCHER†, A. APPERT-FLORY†

Int. Jnl. Lab. Hem. 2016, **38**, 389–396

An Analysis of the Sensitivity of the Activated Partial Thromboplastin Time (APTT) Assay, as Used in a Large Laboratory Network, to Coagulation Factor Deficiencies

Louis Do, BAppSc, MBBS ✉, Emmanuel Favaloro, PhD, FFSC, Leonardo Pasalic, MBBS, PhD

American Journal of Clinical Pathology, Volume 158, Issue 1, July 2022, Pages 132–141,

THE SENSITIVITY OF TWO NEW APTT REAGENTS TO FACTORS VIII, IX AND XI

A. Bowyer, S. Kitchen and R. Maclean

Department of Coagulation, Sheffield Haemophilia and Thrombosis Centre, UK

ISTH 2020 VIRTUAL CONGRESS JULY 12-14

APTT REAGENT	COMPOSITION	SENSITIVITY (IU/dL)		
	EA –ellagic acid RB-rabbit brain PL-phospholipid	FVIII	FIX	FXI
Yumizen G APTT 4	Micronised silica RB PL	40.9	26.8	43.3
Yumizen G APTT Liq 4	EA, RB PL	37.4	29.7	48.9
Actin FS	EA purified soy phosphatides	67.3	25.5	49.3
Actin FSL	EA soy/RB phosphatides	46.7	22.6	28.3
APTT SP	Silica synthetic PL	38.4	15.9	35.6
STA-PTTA	Silica, RB PL	47.6	29.0	35.7
Synthafax	EA synthetic PL	62.8	27.5	55.2
Synthasil	Silica synthetic PL	31.5	36.1	49.0

KEY POINT

A normal APTT does not necessarily indicate normal coagulation

Date	PT/INR	APTT ratio	APTTr mix	TT ratio	Fib. g/L
	0.8 – 1.2	0.8 – 1.2	0.8 – 1.2	0.85 – 1.15	1.7 – 3.9
04.05.18	1.0	**1.4**	1.0	0.92	-
05.05.18	1.0	**1.3**	-	-	-
07.05.18	1.0	1.1	-	-	-
08.05.18	1.1	1.0	-	-	-
10.05.18	1.0	1.2	-	-	-
13.05.18	1.0	**1.3**	-	-	-
14.05.18	1.0	**1.5**	-	-	-
15.05.18	1.0	1.2	-	0.95	6.4

Lupus anticoagulant assays (15.05.18)			
Assay		**Result**	**RR**
dRVVT screen	(ratio)	**1.32**	0.85 – 1.17
dRVVT confirm	(ratio)	1.10	0.90 – 1.10
% correction		**16.7**	≥ 10
dRVVT screen 50:50 mix	(ratio)	**1.11**	0.90 – 1.07
dRVVT confirm 50:50 mix	(ratio)	0.99	0.98 – 1.10
dAPTT screen	(ratio)	0.98	0.80 – 1.20

43 year old male

Renal transplant in 2006

Admitted for renal surgery May 2018

No history of bleeding

LA detected by dRVVT but not dilute APTT

Is the LA causing fluctuating elevation of APTT?

FXII deficiency masked by grossly elevated FVIII (± high fibrinogen)

FII:C	(iu/dL)	107	50 – 150
FV:C	(iu/dL)	149	50 – 150
FVII:C	(iu/dL)	83	50 – 150
FX:C	(iu/dL)	125	50 – 150
FVIII:C	(iu/dL)	**536**	50 – 150
FIX:C	(iu/dL)	111	50 – 150
FXI:C	(iu/dL)	99	58 – 120
FXII:C	(u/dL)	**27**	50 – 150

blood

Bleeding in carriers of hemophilia

Iris Plug, Eveline P. Mauser-Bunschoten, Annette H. J. T. Bröcker-Vriends, Hans Kristian Ploos van Amstel, Johanna G. van der Bom, Joanna E. M. van Diemen-Homan, José Willemse, and Frits R. Rosendaal

Blood. 2006;108:52-56

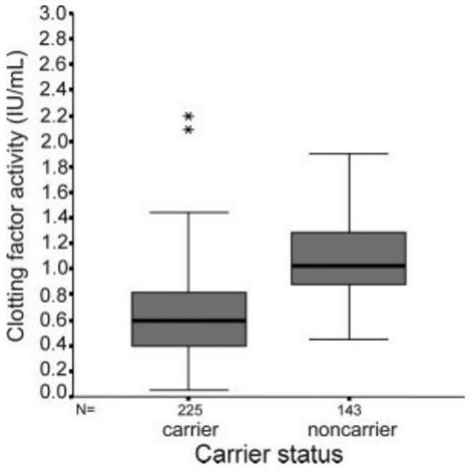


Figure 1. Clotting factor level in relation to carrier status shown for participants for whom clotting factor level is known. This box-whisker plot shows the median and the interquartile range of clotting factor activity levels in carriers and noncarriers. The box is marked by the first and the third quartile; the whiskers indicate the range. The stacked asterisks indicate two extremes (2.09 and 2.19 IU/mL).

APTT REAGENT	COMPOSITION	SENSITIVITY (IU/dL)		
		FVIII	FIX	FXI
Yumizen G APTT 4	Micronised silica RB PL	40.9	26.8	43.3
Yumizen G APTT Liq 4	EA, RB PL	37.4	29.7	48.9
Actin FS	EA purified soy phosphatides	67.3	25.5	49.3
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Synthafax	EA synthetic PL	62.8	27.5	55.2
Synthasil	Silica synthetic PL	31.5	36.1	49.0

Normal APTT

Table 3. Bleeding tendency of both carriers and noncarriers according to decreasing clotting factor level

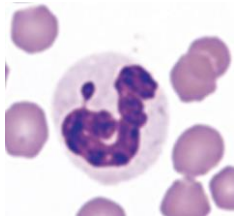
	Clotting factor level			
	More than 0.60 IU/mL	Between 0.41 and 0.60 IU/mL	0.40 IU/mL or below	*P* for trend
Small wounds				< .01
Event/total (%)	28/233 (12)	25/64 (39)	11/60 (18)	
RR (CI)	1	3.3 (2.0-5.2)	1.5 (0.8-2.9)	
Joint bleeds				.06
Event/total (%)	12/241 (5)	9/65 (14)	6/62 (10)	
RR (CI)	1	2.8 (1.2-6.3)	1.9 (0.8-4.9)	
Tonsillectomy				.06
Event/total (%)	21/124 (17)	6/26 (23)	11/31 (35)	
RR (CI)	1	1.4 (0.6-3.0)	2.1 (1.1-3.9)	
Tooth extraction				< .01
Event/total (%)	18/139 (13)	14/51 (27)	15/36 (42)	
RR (CI)	1	1.8 (1.0-3.0)	2.5 (1.5-4.2)	
Operations				< .01
Event/total (%)	18/139 (13)	14/49 (29)	15/36 (42)	
RR (CI)	1	2.2 (1.2-4.1)	3.2 (1.8-5.7)	
Bleeding score 2 or above, RR (CI)	1	3.0 (1.5-5.8)	4.0 (2.1-7.7)	< .01

Women who ever received treatment with clotting factor concentration, tranexamic acid, or desmopressin before tooth extraction, tonsillectomy, or operations were excluded from the analysis.

Haemophilia in females

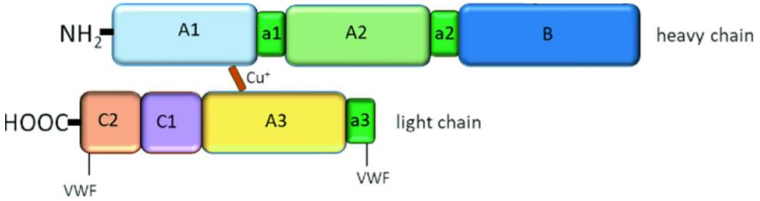
Some carriers have sufficient reduction in FVIII/FIX to have bleeding symptoms and may require treatment prior to invasive procedures or after major trauma

Reduced FVIII levels in females



- Extreme lyonisation in a carrier
- Hemizygosity (i.e. XO in Turner Syndrome)
- Type 2N or severe VWD
- True haemophiliac female
- Acquired haemophilia

Assay discrepant non-severe Haemophilia A



Clinically significant discrepancies between 1-stage assay (OSA) & chromogenic substrate assay (CSA)

Approximately 30% of non-severe HA

Classical discrepancy – higher OSA result

Unstable FVIIIa heterotrimer and increased A2 dissociation

Clinical severity more in keeping with the lower 2-stage assay result

Reverse/Inverse discrepancy - higher CSA result

Impaired activation of FVIII by thrombin

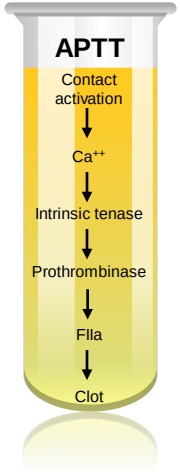
Clinical severity more in keeping with the higher 2-stage result (bleeding rare)

FVIII:Ag often normal or near normal

Classical discrepancy
(Higher 1-stage)

FVIIIa formed only in final, rapid stage of clotting assay

Effects of **unstable FVIIIa** minimised

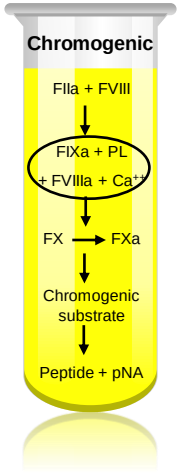


Inverse discrepancy
(Higher 2-stage i.e. CSA)

Impaired activation of FVIII by thrombin

Variants activated by thrombin but at a slower rate

Short FVIII activation period leads to reduced result



Prolonged incubation with FIIa

Higher A2 dissociation rate leads to lower result

Higher dilution further influences high dissociation rate

Impaired thrombin cleavage compensated by:

- supraphysiological thrombin & FIX concs
- longer incubation
- diluted FVIII protein

Prevalence, biological phenotype and genotype in moderate/mild hemophilia A with discrepancy between one-stage and chromogenic factor VIII activity

M. TROSSAËRT,* P. BOISSEAU,† A. QUEMENER,‡ M. SIGAUD,* M. FOUASSIER,* C. TERNISIEN,* A. LEFRANÇOIS-BETTEMBOURG,* C. TESSON,† C. THOMAS† and S. BEZIEAU†

J Thromb Haemost 2011; **9**: 524–30.

Most patients with reverse discrepancy will have an elevated APTT

BUT

Rarely bleed because 2-stage assay better reflects clinical severity

Many patients with classical discrepancy will have a normal APTT

BUT

- More likely to bleed as 2-stage assay better reflects clinical severity
- Diagnosis could be missed from normal APTT &/or normal 1-stage

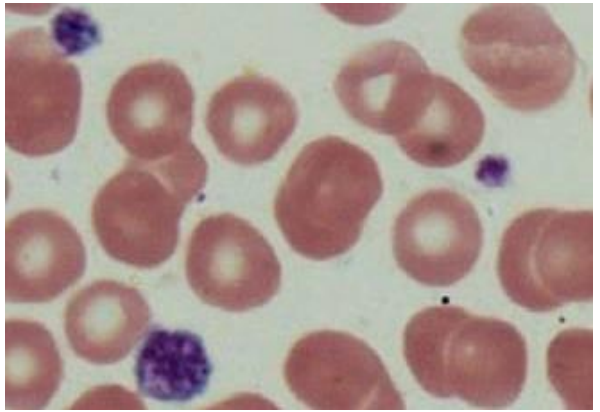
	Mutation		FVIII:C			
Family	HAMSTeRS	HGVS	1-st assay (IU dL^{-1})	Chromogenic assay (IU dL^{-1})	1-st FVIII:C/ Chromo-FVIII:C ratio	FVIII:Ag (IU dL^{-1})
Low ratio						
A	Phe2127Ser	p.Phe2146Ser	7	49	0.14	71
			5	31	0.16	41
			20	100	0.20	148
			14	64	0.22	84
			16	72	0.22	94
			12	53	0.23	61
			8	33	0.24	30
			14	52	0.27	49
			24	67	0.36	70
			13	51	0.25	50
B	Phe2127Ser	p.Phe2146Ser	4	32	0.13	50
			4	30	0.13	16
C	Phe2127Ser	p.Phe2146Ser	10	55	0.18	34
			25	124	0.20	130
D	Phe2127Ser	p.Phe2146Ser	8	40	0.20	43
			13	45	0.29	58
E	Phe2127Ser	p.Phe2146Ser	18	76	0.24	82
			16	57	0.28	57
F	ND	ND	18	61	0.30	74
G	Ile369Thr	p.Ile388Thr	10	82	0.12	126
			9	74	0.12	91
			20	105	0.19	156
			16	100	0.16	100
H	Phe2127Ser	p.Phe2146Ser	9	32	0.28	47
			7	18	0.39	39
I	Glu720Lys	p.Glu739Lys	30	99	0.30	128
J	Phe2127Ser	p.Phe2146Ser	3	11	0.27	18
			4	14	0.29	26
K	ND	ND	39	194	0.20	195
L	Glu720Lys	p.Glu739Lys	39	115	0.34	144
High ratio						
M	His281Asn	p.His300Asn	38	25	1.52	70
N	Arg531His	p.Arg550His	81	39	2.08	97
O	Arg1749His	p.Arg1768His	71	32	2.22	80
P	Phe1785Leu	p.Phe1804Leu	21	13	1.62	40
R	Pro264Leu	p.Pro283Leu	30	16	1.88	47
			14	5	2.80	39


Platelets

21 yr old Zimbabwean woman admitted with GI bleed requiring transfusion

Long history of epistaxis, haematemesis & menorrhagia; bled after appendectomy at 9 yrs old - multiple transfusions

Previous presumptive diagnosis of ITP but poor response to corticosteroids



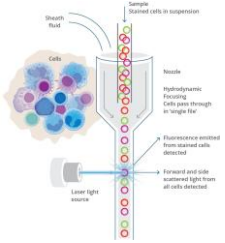
Macrothrombocytopenia

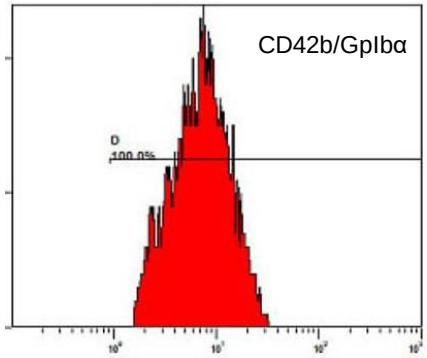
VWF:RCo, VWF:Ag & VWF:CB all normal

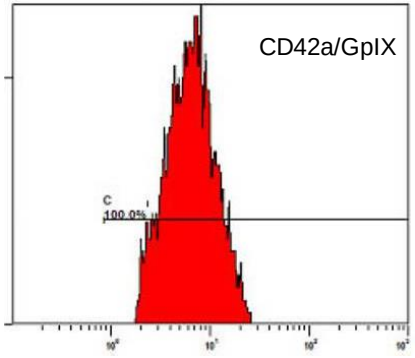
Platelet count: 69 x 10^9/L

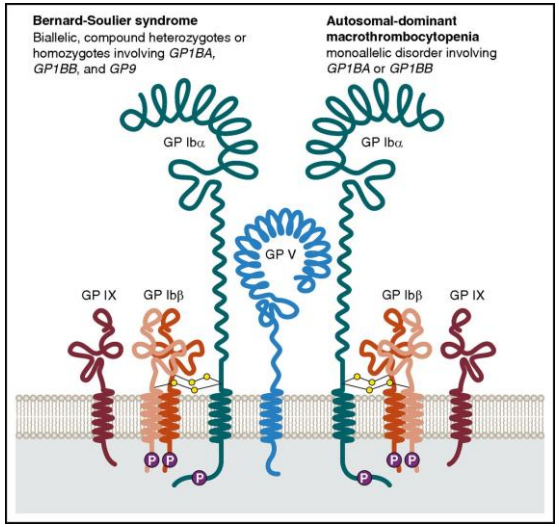
Aggregometry: normal with ADP, epinephrine, collagen, TRAP, U46619
markedly reduced with ristocetin

Flow cytometry









Homozygous for c.488C>A substitution (p.Ala163Asp) within exon 3 of *GP9*

Low frequency polymorphism restricted to sub-set of African population

Only heterozygous individuals reported previously (not presenting as BSS)

Normal quantitative expression of GpIb-IX-V complex

Functional defect leading to identical phenotype to classical BSS

Haemophilia
The Official Journal of the World Federation of Hemophilia, European Association for Haemophilia and Allied Disorders and the Hemostasis & Thrombosis Research Society

A diagnostic dilemma: variant Bernard–Soulier syndrome, a difficult clinical and genetic diagnosis

S. OKOLI,* B. MADAN,† A. MWIRIGI,* G. MOORE,* A. DREW,* M. J. MITCHELL‡ and J. A. CUTLER† ‡
*Haematology; †Haemophilia, Guys & St Thomas NHS foundation Trust; and ‡Molecular Haemostasis, Viapath LLP St Thomas' Hospital, London, UK

Bernard–Soulier syndrome is a rare autosomal recessive bleeding disorder, with an estimated frequency of 1:1 000 000, clinically characterized by mucocutaneous bleeding [1]. The classic laboratory findings are macrothrombocytopenia, absent ristocetin-induced platelet aggregation and absent or markedly reduced Glycoprotein Ib-IX-V expression on platelet surfaces. Genetic abnormalities have been identified in three of the genes encoding the subunits of the GPIb-IX-V complex, *GP1bA*, *GP1bB* and *GP9* [2]. No causative mutations of Bernard–Soulier syndrome have been reported in *GP5*, and this is consistent with a lack of requirement for GPV expression for expression of the other subunits of the GPIb-IX-V complex.

This case reports a young patient with a complex clinical history, in whom non-classical phenotypic and genotypic laboratory findings complicate the diagnosis of Bernard–Soulier syndrome.

A 21-year-old Zimbabwean female presented with a long history of epistaxis, haematemesis and menorrhagia. Her medical history included migraine, epilepsy and asthma, and she has a single duplex kidney. The patient reports excessive bleeding on previous surgical challenge – bleeding post appendectomy, at 9 years old, requiring multiple blood transfusion and return to theatre for evacuation of haematoma. She has no significant family history, and her parents are non-consanguineous. The patient had extensive ENT and gastrointestinal investigations, which were negative, and was given a presumptive diagnosis of ITP, though importantly she was poorly responsive to corticosteroids.

On this admission she presented a 5-day history of severe acute abdominal pain, haematuria and significant gastrointestinal bleeding requiring blood transfusion. Clinical examination indicated no skeletal abnormalities, normal neurocognitive and cardiac assessments with no hearing deficit. Initial laboratory findings showed platelets of 69×10^9 per L and macrothrombocytopenia with no neutrophilic inclusions (Fig. 1).

Standard coagulation and von Willebrand factor screens were normal. Further investigations revealed a marked reduced aggregation in response to ristocetin at 0.25 g L^{-1} (Fig. 2). Platelet glycoprotein expression studies showed normal levels of glycoproteins Ib, and IX, and prompted molecular analysis to confirm the diagnosis of Bernard–Soulier syndrome. All exons and splice junctions of the *GP1bA*, *GP1bB* & *GP9* genes were subjected to PCR amplification and sequencing, and the only variant detected was a homozygous c.488C>A substitution within exon 3 of *GP9*.

The patient underwent further urological and gastrointestinal investigations which were once again unremarkable. She was commenced on tranexamic acid and transfused HLA matched platelets, where appropriate. Her bleeding ceased after 14 days, and she was discharged home.

The c.488C>A substitution (Fig. 3) predicts the replacement of the native alanine residue at codon

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Tel.: +02071887188; fax: +02071882728;
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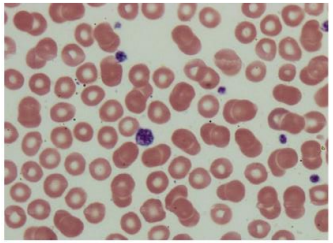


Fig. 1. Peripheral blood film – showing macrothombocytes in variant Bernard–Soulier syndrome.


Thrombophilia

30 year old male

Unprovoked DVT

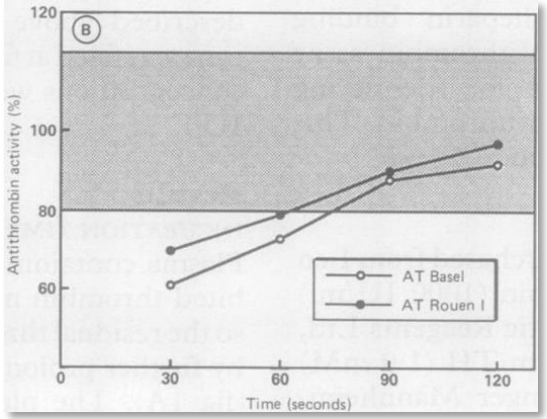
Local hospital antithrombin activity (FXa inhibition):	75%	(RR: 80 – 125%)
Reference lab. antithrombin activity (FXa inhibition):	28%	(RR: 85 – 120%)
Local lab. heparin-binding incubation period:	3 minutes	
Reference lab. heparin-binding incubation period:	30 seconds	

Journal of Clinical Pathology

Screening for heparin binding variants of antithrombin

P L Harper, M Daly, J Price, P F Edgar, R W Carrell

J Clin Pathol 1991;44:477–479



RECOMMENDATIONS AND GUIDELINES

jth

Recommendations for clinical laboratory testing for antithrombin deficiency; Communication from the SSC of the ISTH

Elizabeth M. Van Cott[1] | Christelle Orlando[2] | Gary W. Moore[3] | Peter C. Cooper[4] | Piet Meijer[5] | Richard Marlar[6] | for the Subcommittee on Plasma Coagulation Inhibitors

J Thromb Haemost. 2020;18:17–22.

3. The recommended initial test for antithrombin deficiency is a chromogenic activity (functional) assay with heparin and preferably a short incubation time of 30 seconds, unless reagents are proven to detect HBS mutations with longer incubation times.

Progressive antithrombin assay

Progressive chromogenic anti-factor Xa assay and its use in the classification of antithrombin deficiencies

Bettina Kovács, Zsuzsanna Bereczky, Anna Selmeczi, Réka Gindele, Zsolt Oláh, Adrienne Kerényi, Zoltán Boda and László Muszbek*

Clin Chem Lab Med 2014;52:1797-1806

Heparin-Antithrombin Binding Ratio

Development of a novel, rapid assay for detection of heparin-binding defect antithrombin deficiencies: the heparin-antithrombin binding (HAB) ratio

Gary W. Moore *, Naomi de Jager, Jacqueline A. Cutler

Thrombosis Research 135 (2015) 161–166

2-dimensional crossed immunoelectrophoresis

Antithrombins Southport (Leu 99 to Val) and Vienna (Gln 118 to Pro): two novel antithrombin variants with abnormal heparin binding

V. Chowdhury,[1] B. Mille,[2] R. J. Olds,[1] D. A. Lane,[2] J. Watton,[3] T. W. Barrowcliffe,[3] I. Pabinger,[4] B. E. Woodcock[5] and S. L. Thein[1]

British Journal of Haematology, 1995, **89**, 602–609

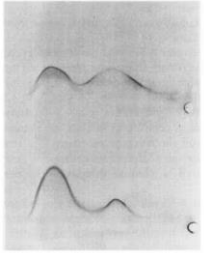


Fig 1(b). Crossed immunoelectrophoresis, with heparin in the first dimension, of plasma from propositus III and a normal control.

36 year old male

Proximal DVT after trauma and immobilisation, previous DVT & PE after long distance flight at age 31

Aunt with history of DVT

Assay		Result	RR
Antithrombin activity	(IU/dL)	105	80 - 120
Protein C activity	(IU/dL)	106	75 - 125
Free protein S antigen	(IU/dL)	115	72 - 130
APC-R (Modified APTT)	(ratio)	2.9	2.0 – 3.5

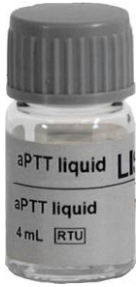
Assay		Result	RR
Antithrombin activity	(IU/dL)	101	79 - 124
Protein C activity	(IU/dL)	**49**	70 – 140
Protein C antigen	(IU/dL)	120	60 - 125
Free protein S antigen	(IU/dL)	97	76 - 129
APC-R (Modified APTT)	(ratio)	3.1	<2.0

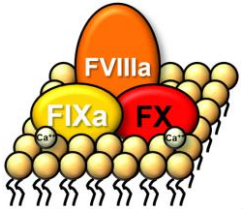
Type 2b PC deficiency

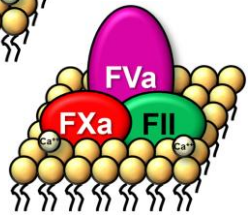
Reduced activity
Normal antigen

Protein C activity assays

Agkistrodon contortrix protein C activator





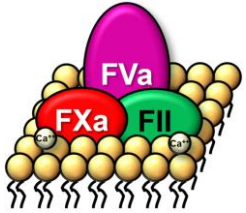


Protein C

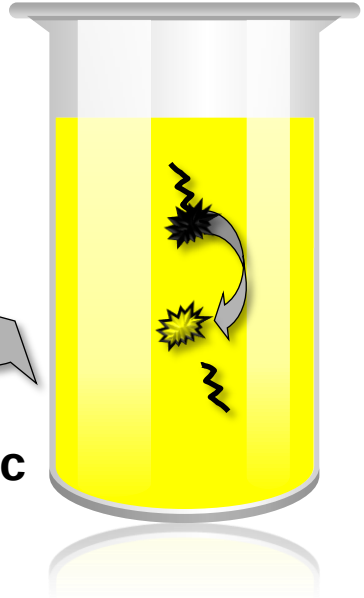
APC

Anticoagulate clotting test

RVVT



Cleave chromogenic substrate



Chromogenic assay

Reliable assays

Not affected by other coagulopathies

Detects abnormalities of:
- protein C activation
- enzymatic active site

Clotting assay

Interferences:
- coagulopathies, LA & high FVIII
- therapeutic anticoagulation
- activated protein C resistance

Detects abnormalities of:
- protein C activation
- enzymatic active site
- FVa & FVIIIa binding
- protein S binding
- phospholipid binding

Southern copperhead
(*Agkistrodon c. contortrix*)

RECOMMENDATIONS AND GUIDELINES

jth

Recommendations for clinical laboratory testing for protein C deficiency, for the subcommittee on plasma coagulation inhibitors of the ISTH

Peter C. Cooper[1] | Anna Pavlova[2] | Gary W. Moore[3] | Kieron P. Hickey[1] | Richard A. Marlar[4]

J Thromb Haemost. 2020;18:271–277.

TABLE 1 Classification of protein C deficiency, and sensitivity and specificity of routinely available assays

Protein C assays and classification according to assay results					
Assay type and characteristics		Type 1 deficiency	Type 2 deficiency		Risk from interferences
			Subtype 2a	Subtype 2b	
Chromogenic Detects enzymatic cleavage of a small synthetic substrate	Venom	✓	✓	✗	Activated samples, HIL
Clotting-based Detects cleavage of FV and FVIII (Venom-APTT) or FV (Venom-RVV); both require co-factors, including PL, Ca^{2+}, PS	Venom-APTT	✓	✓	✓	Activated samples, LA, high FVIII, APC-R, DOAC, high level of heparin/low molecular weight heparin, HIL
	Venom-RVV	✓	✓	✓	Activated samples, LA, APC-R, DOAC, high level of heparin/low molecular weight heparin, HIL
Antigen	RID/IE	✓	✗	✗	EDTA required in IE
Quantitative assay, does not detect PC function	ELISA ELFA	✓	✗	✗	Nonspecific binding, HIL

Laboratory Limitations of Excluding Hereditary Protein C Deficiency by Chromogenic Assay: Discrepancies of Phenotype and Genotype

Clinical and Applied Thrombosis/Hemostasis
Volume 26: 1-13

DOI: 10.1177/1076029620912028
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SAGE

Holger Seidel, MD[1], Bianca Haracska, BSc[1], Jennifer Naumann, PhD[1], Philipp Westhofen, PhD[1], Moritz Sebastian Hass, MSc[1], and Johannes Philipp Kruppenbacher, MD[1]

Table 2. Characteristics of Patients With and Without PC Variation.

Patient Characteristics	Included Patients (n = 287)		
	Patients: PC Variation, Chromogen PC Activity ≥70%	Patients: PC Variation, Chromogen PC Activity <70%	Patients: Without PC Variation
Number of patients	20	81	186
Females	14	47	154
Males	6	34	32
Age (years)	40.9 ± 19.2	36.8 ± 16.8	37.1 ± 15.5
PC activity chromogen (%)	80.5 ± 11.3	50.8 ± 11.2	77.3 ± 14.7
PC activity clotting (%)	55.9 ± 10.2	44.0 ± 12.0	62.7 ± 15.8
PC antigen (%)	69.9 ± 14.8	48.4 ± 16.2	65.3 ± 14.1
PC antigen (mg/L)	2.4 ± 0.3	1.9 ± 0.6	2.4 ± 0.3
F VIII activity (%)	116.3 ± 40.6	114.9 ± 37.2	135.7 ± 53.8
Lupus anticoagulant (lac screen ratio)	1.3 ± 0.5	1.0 ± 0.3	1.0 ± 0.2
Deep vein thrombosis	7 (35%)	26 (32%)	64 (34%)
Pulmonary embolism	3 (15%)	8 (10%)	21 (11%)
Factor V Leiden	6 (30%)	10 (12%)	44 (24%)
Women with pregnancy complications	2 (10%)	9 (19%)	42 (27%)


Inhibitors

Diabetic 66 year old female

Recent ciprofloxacin & penicillin for painful, oozing leg ulcers

Presented with melena: Hb 60 g/L

INR >10 APTTr 4.0 FII:C very low (!)

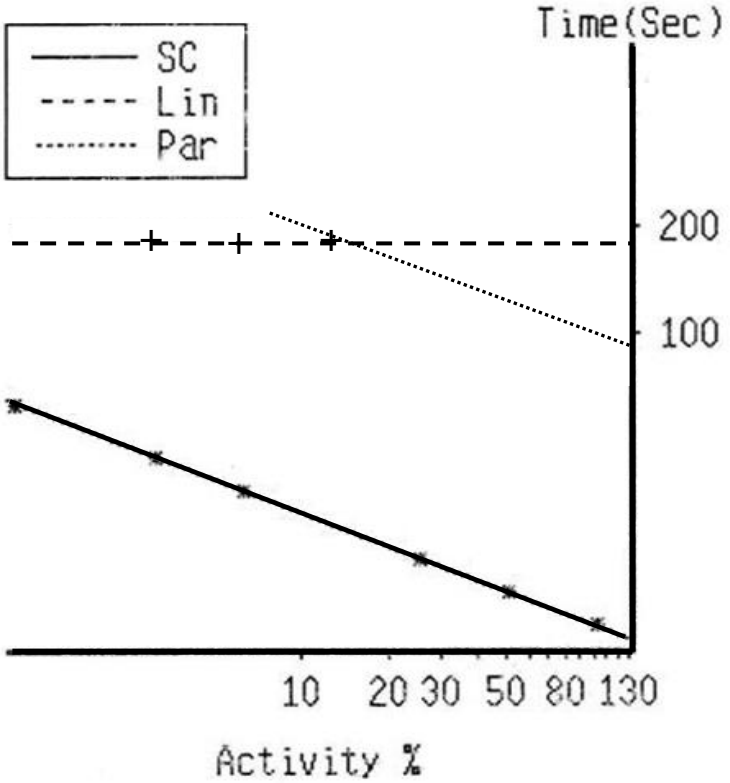
Patient denies taking warfarin - given vitamin K but no correction (yet)

Assay		Result	RR
PT/INR on photo-optical analyser (recombinant human thromboplastin) 		**>10**	0.8 – 1.2
PT/INR manual with mechanical clot detection (rabbit brain thromboplastin) 		**7.0**	0.8 – 1.2
APTT on analyser	(ratio)	**>10**	0.8 – 1.2
APTT (manual)	(ratio)	**4.8**	0.8 – 1.2
TT	(ratio)	1.15	0.80 – 1.23
Fibrinogen	(g/L)	3.0	2.0 – 4.0

Assay		Result	RR
Plasma warfarin	(mg/L)	**< 0.12**	0.70 – 2.30
Vitamin K1	(µg/L)	**11.0**	0.15 – 1.55
Vitamin K epoxide	(µg/L)	**<0.12**	0.00 – 0.12

All one-stage PT & APTT based assays flat-lined at dilutions of 1/10, 1/20, 1/40

FVIII by CSA
399 IU/dL



II MDA

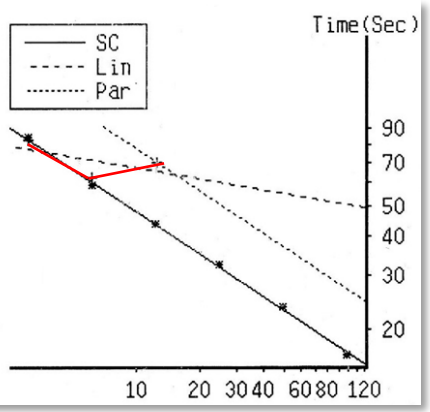
MDA Ratio	Clot time	Activity %
1/ 8	69.5 sec	36.6 iu/d
1/16	62.4 sec	92.6 iu/d
1/32	82.2 sec	102.2 iu/d

Sample dilutions
1/80
1/160
1/320

Mean 77.1 iu

SCr= -0.999 Test r= -0.604

SR= 0.26



FII inhibition almost abolished at high dilutions
>102 IU/dL

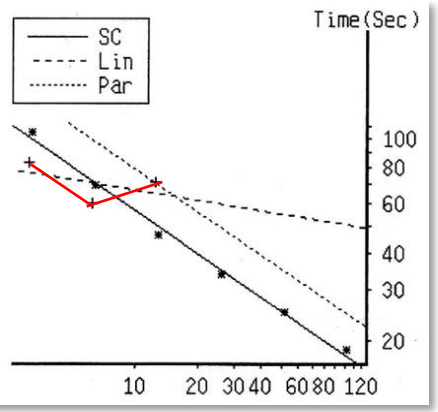
VII MDA

MDA Ratio	Clot time	Activity %
1/ 8	70.3 sec	51.0 iu/d
1/16	59.6 sec	141.9 iu/d
1/32	83.0 sec	146.4 iu/d

Mean 113.1 iu

SCr= -0.997 Test r= -0.501

SR= 0.24



FVII inhibition abolished at high dilutions
144.2 IU/dL

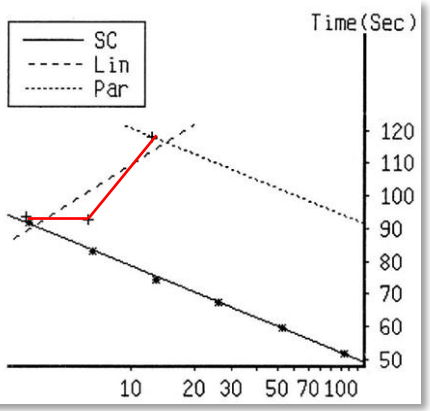
IX MDA

MDA Ratio	Clot time	Activity %
1/ 8	118.2 sec	2.8 iu/d
1/16	92.8 sec	46.3 iu/d
1/32	93.6 sec	86.4 iu/d

Mean 45.2 iu

SCr= -0.999 Test r= 0.852

SR= -1.56



FIX inhibition not fully abolished at high dilutions
>86.4 IU/dL

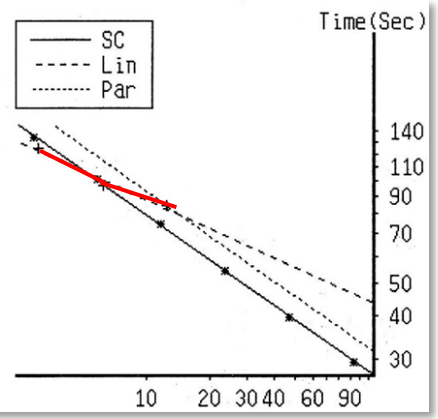
X MDA

MDA Ratio	Clot time	Activity %
1/ 8	84.4 sec	69.6 iu/d
1/16	96.8 sec	102.1 iu/d
1/32	124.8 sec	115.4 iu/d

Mean 95.7 iu

SCr= -1.000 Test r= -0.985

SR= 0.64



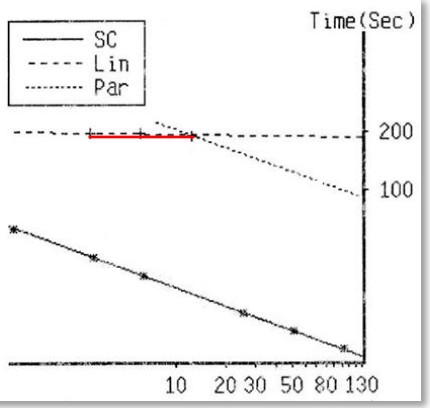
FX inhibition almost abolished at high dilutions
>115.4 IU/dL

V MDA

MDA Ratio	Clot time	Activity %
1/ 8	190.0 sec	0.6 iu/d
1/16	194.0 sec	1.2 iu/d
1/32	194.0 sec	2.5 iu/d
Sample dilutions		
1/80		
1/160		
1/320		
		Mean 1.4 iu

SCr= -1.000 Test r= -0.866

SR= 0.05



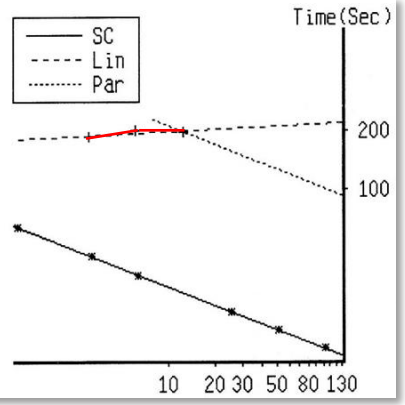
FV ostensibly still flatlining
level rising with dilution (beyond std curve)

V MDA

MDA Ratio	Clot time	Activity %
Sample dilutions		
1/320	196.0 sec	0.6 iu/d
1/640	198.0 sec	1.2 iu/d
1/1280	186.0 sec	2.7 iu/d
		Mean 1.5 iu

SCr= -1.000 Test r= 0.780

SR= -0.12



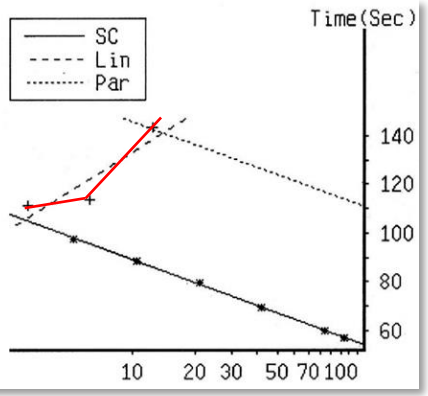
FV ? inhibition being diluted ? dilution too high
level rising with dilution (beyond std curve)

XI MDA

MDA Ratio	Clot time	Activity %
1/ 8	143.2 sec	1.9 iu/d
1/16	113.4 sec	27.9 iu/d
1/32	111.0 sec	66.3 iu/d
		Mean 32.0 iu

SCr= -1.000 Test r= 0.898

SR= -1.70



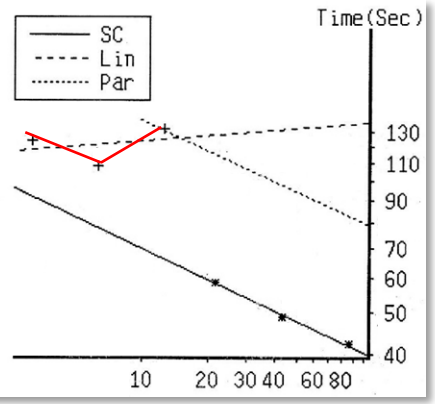
FXI inhibition not fully abolished at high dilutions
? >66.3 IU/dL (beyond standard curve)

XII MDA

MDA Ratio	Clot time	Activity %
1/ 8	131.4 sec	6.0 iu/d
1/16	108.6 sec	25.9 iu/d
1/32	124.2 sec	30.1 iu/d
		Mean 20.7 iu

SCr= -0.999 Test r= 0.288

SR= -0.17



FXII inhibition almost abolished at high dilutions
? >30.1 IU/dL (beyond standard curve)

Antiphospholipid antibodies

LA assays			RR
dRVVT	(ratio)	**No clot**	0.86 – 1.19
dRVVT confirm	(ratio)	**No clot**	0.83 – 1.13
dRVVT screen 50:50 mix	(ratio)	**7.52**	0.90 – 1.10
dRVVT confirm 50:50 mix	(ratio)	**No clot**	0.94 – 1.13
dAPTT	(ratio)	**No clot**	0.81 – 1.23
dAPTT confirm	(ratio)	**No clot**	0.81 – 1.13
dAPTT screen 50:50 mix	(ratio)	**4.98**	0.86 – 1.15
dAPTT confirm 50:50 mix	(ratio)	**4.24**	0.85 – 1.09
% correction of screen by confirm		**14.9**	<10%
Solid-phase assays			
IgG aCL	(GPL U/mL)	2.6	>7.0
IgM aCL	(MPL U/mL)	1.4	>7.0
IgG antiprothrombin antibodies	(U/mL)	3.5	>6.0
IgM antiprothrombin antibodies	(U/mL)	2.8	>5.0

? extremely potent lupus anticoagulant due to dAPTT mixing tests

.....yet dRVVT confirmatory tests overwhelmed

Snake venom prothrombin activators

Assay	Result	RR
Taipan snake venom time (ratio)	**1.29**	0.91 – 1.11
Ecarin time (ratio)	0.96	0.92 – 1.08
% correction	**25.6**	<10

Venom fraction	Co-factor requirements
Oscutarin C	Phospholipid Ca^{2+}
Ecarin	None

FV Bethesda assay	**307 BU/mL**

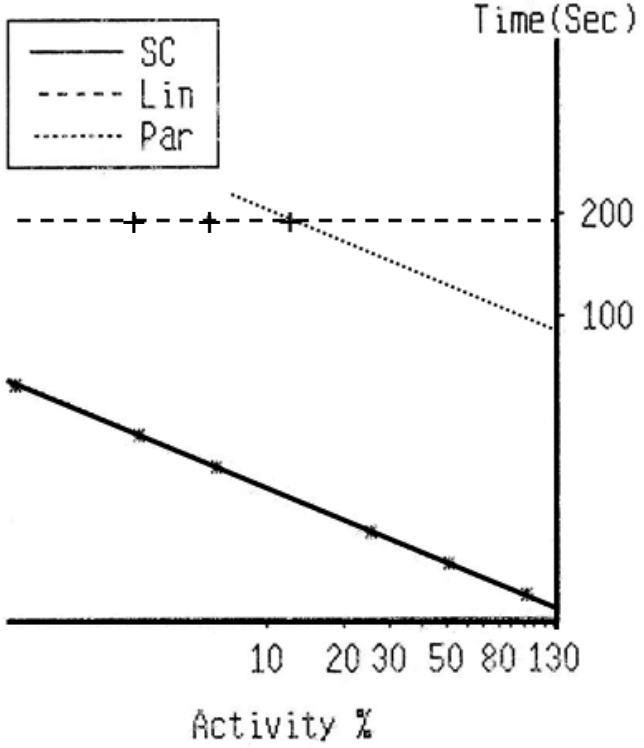
V MDA

MDA Ratio	Clot time	Activity %
1/ 8	190.0 sec	0.6 iu/d
1/16	194.0 sec	1.2 iu/d
1/32	194.0 sec	2.5 iu/d
		Mean 1.4 iu

Sample dilutions:
1/80
1/160
1/320

SCr= -1.000 Test r= -0.866

SR= 0.05



Although activity apparently rises with dilution, each clotting time is effectively a blank

Bleeding in the antiphospholipid syndrome

Ricardo Forastiero

Hematology 2012 VOL. 17 SUPPL. 1 S153

- Thrombocytopenia rarely severe enough to cause bleeding
  - except CAPS, DIC
- Lupus anticoagulant hypoprothrombinemia syndrome

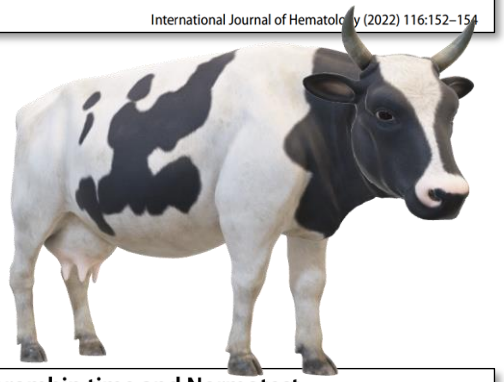
Lupus anticoagulant-hypoproaccelerin (factor V) syndrome (LAHPS-V): a new hemorrhagic condition associated with lupus anticoagulant

Masahiro Ieko[1,2,3] · Sumiyoshi Naito[4] · Mika Yoshida[4] · Kazumasa Ohmura[3] · Nobuhiko Takahashi[3] · Norifumi Sugawara[1] · Kazuki Kiyohara[1] · Kenji Shimosegawa[1] · Akitada Ichinose[5]

International Journal of Hematology (2022) 116:152–154

Table 1 Cases of coagulation factor V inhibitor with lupus anticoagulant and the suspicious cases

No	Age (years)	Sex	Underlying conditions	Symptoms	FV:C (%)	FV-inhibitor (BU/mL)	LA	Remarks
(A) Definite cases								
A-1	71	M	Temporo-parietal astrocytoma	Left popliteal vein thrombosis	<3.0	61	dRVVT positive	Positive for aCL-IgG and -IgM
A-2	29	M	Appendicular infiltrate, antibiotics	No symptoms (abnormal coagulation tests)	23	1.0	dRVVT positive	
A-3	71	F	Hemangiopericytoma, UTI, antibiotics	Left femoral DVT	1	1.4	dRVVT positive Staclot LA positive	
A-4	58	M	Unknown	DVT	8	2.65	dRVVT positive	
A-5	71	F	Proctoscopy, antibiotics	Ecchymosis, hematoma	<2.0	6.0	PNP positive, dRVVT: NC	
A-6	63	M	Lithotripsy, antibiotics	Hematuria	2	16	PNP positive, dRVVT: NC	
A-7	76	F	Pneumonia, antibiotics	Subdural hematoma	2	70	dRVVT positive	
A-8	71	F	Pancreatic cancer	Multiple purpura	<3.0	1.7	dRVVT positive	
A-9	71	F	HT, HL, stroke	Petechiae, ecchymoses, melena	<3.0	8.0	dRVVT positive	Positive for aCL-IgG
A-10	56	M	HT, DM	Purpura, hematuria	2.3	1.9*	Staclot LA positive	
A-11	52	F	SLE, DM	Multiple purpura	2.5	3.0*	Staclot LA positive	
(B) Suspicious cases								
B-1	92	F	HT, HF, Af, PE, erysipelas, leg infection, antibiotics	Extensive hematomas	1	496*	dRVVT: NC	
B-2	87	M	DM	Recurrent bleeding from the hemodialysis puncture site	<1	415*	dRVVT: NC	
B-3	70	M	Unknown	Subcutaneous hemorrhage, right thigh hematoma	12.4	NR**	dRVVT: NC	Positive for aCL



A discrepancy between prothrombin time and Normotest (Hepaplastintest) results is useful for diagnosis of acquired factor V inhibitors

Yasuko Kadohira[1] · Shinya Yamada[1] · Tomoe Hayashi[1] · Eriko Morishita[1] · Hidesaku Asakura[1,2] · Akitada Ichinose[2,3]

International Journal of Hematology (2018) 108:145–150

- Normotest contains rabbit brain thromboplastin adsorbed bovine plasma
- Even if Normotest result abnormal, bovine FV provides compensatory effect relative to plain thromboplastin

16 yr old girl and her 41 yr old father

She bled profusely after surgery

Father history of haematoma development after trauma (began at 14)

Looks like UFH but not anticoagulated

dRVVT might suggest LA

Why the incoagulable thrombin times?

There is certainly an inhibitor

Assay		Daughter	Father	RR
PT	(s)	**19.2**	**17.2**	11.5 – 15.5
PT 50:50 mix	(s)	13.9	13.6	11.5 – 15.5
APTT	(s)	**>180**	**>180**	28.0 – 43.5
APTT 50:50 mix	(s)	**99.9**	**73.2**	28.0 – 43.5
TT	(s)	**>240**	**>240**	11.0 – 19.0
TT 50:50 mix	(s)	**>240**	**>240**	11.0 – 19.0
Reptilase time	(s)	16.0	15.9	11.0 – 19.0
Fibrinogen	(g/L)	3.9	2.9	2.0 – 4.0
dRVVT	(s)	**99.8**	**102.3**	27.0 – 41.0
dRVVT 50:50 mix	(s)	**53.5**	**57.6**	27.0 – 41.0
D-dimers	(µg/mL)	0.01	0.07	<0.2

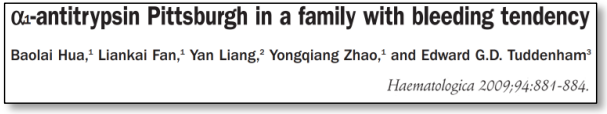
Assay (%)		Daughter	Dad	RR
FII		77	88	50 – 150
FV		95	76	50 – 150
FVII		93	98	50 – 150
FVIII		Normal with increased dilutions	Normal with increased dilutions	50 – 150
FIX		Normal with increased dilutions	Normal with increased dilutions	50 – 150
FX		**36**	**41**	50 – 150
FXI		**2**	**12**	50 – 150
FXII		**20**	**36**	50 – 150
Antithrombin	(FIIa inhibition)	**200**	**190**	89 – 130
Protein C	(chromogenic)	**0**	**0**	69 – 151
Protein S	(APTT)	Normal with increased dilutions	Normal with increased dilutions	52 - 139
Maximal platelet aggregation:	ADP 10µM	**62**	**51**	50 – 100
Maximal platelet aggregation:	FIIa 3 U/mL	**9**	**10**	60 - 100

Looks like another pan-inhibitor - greater influence on APTT-based assays

Remember incoagulable thrombin time but normal reptilase time and fibrinogen

? significance of high antithrombin & reduced thrombin-induced platelet aggregation

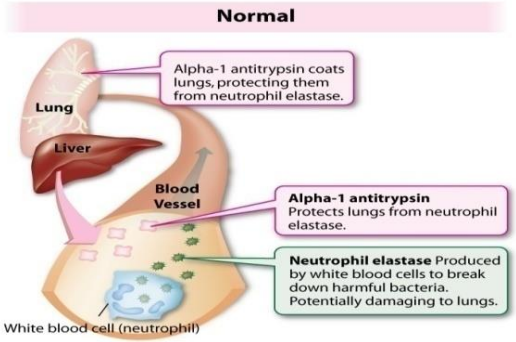
α_1-antitrypsin Pittsburgh

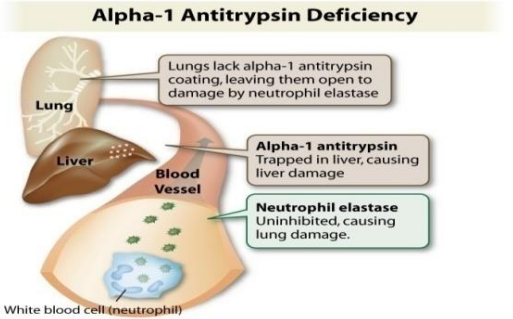

α1-antitrypsin Pittsburgh in a family with bleeding tendency

Baolai Hua,[1] Liankai Fan,[1] Yan Liang,[2] Yongqiang Zhao,[1] and Edward G.D. Tuddenham[3]

Haematologica 2009;94:881-884.

α_1-antitrypsin





α_1-antitrypsin is a SERPIN that inhibits a wide variety of proteases

Protects tissues from enzymes of inflammatory cells, especially neutrophil elastase

Acute phase protein – further elevation limits damage by activated neutrophils

Deficiency leads to chronic, uninhibited tissue breakdown, especially in lungs

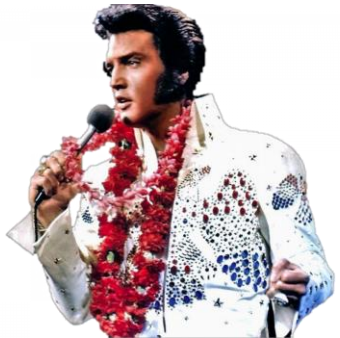
Leads to emphysema & COPD

α_1-antitrypsin Pittsburgh

Bleeding occurs after trauma - acute phase response elevates levels of mutant protein

α_1-antitrypsin Pittsburgh has *increased anti-thrombin activity*

α_1-antitrypsin Pittsburgh has strong affinity for protein C leading to increased turnover and low circulating levels – maintains balance other than in trauma



Key points

Look beyond the obvious

If test results don’t fit the clinical picture, look for the same thing in a different way

56 year old female
Non-consanguineous parents
Asymptomatic

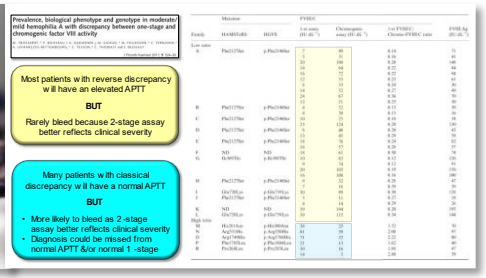
Lab 1			
Assay		Result	RR
PT (s)		22	10 – 13
PT 50:50 mix (s)		12	10 - 13
APTT (s)		38	30 - 40
TT (s)		9	9 - 11
FVII:C	(IU/dL)	120	50 - 150
LA testing by dRVVT, APTT & dPT		Not detected	
FX:C	(IU/dL)	25	50 - 150

Lab 2			
Assay		Result	RR
PT (s)		21	12 – 15
PT 50:50 mix (s)		13	12 - 15
APTT (s)		30	32 – 42
TT (s)		11	10 - 12
FVII:C	(IU/dL)	117	50 - 150
FX:C	(IU/dL)	27	50 - 150

FX Padua

reduced activity with FX chromogenic assay & extrinsic assays
normal activity with Russell's viper venom & intrinsic assays
normal FXAg

Isolated elevated PT expected to be FVII deficiency but was FX deficiency



Haemophilia A hidden by normal APTT due to nature of mutant molecules

Date	PTINR	APTT ratio	APTT mix	TT ratio	Fib. g/L
	0.8 – 1.2	0.8 – 1.2	0.8 – 1.2	0.85 – 1.15	1.7 – 3.9
04.05.18	1.0	1.4	1.0	0.92	-
05.05.18	1.0	1.3	-	-	-
07.05.18	1.0	1.1	-	-	-
08.05.18	1.1	1.0	-	-	-
10.05.18	1.0	1.2	-	-	-
13.05.18	1.0	1.3	-	-	-
14.05.18	1.0	1.5	-	-	-
15.05.18	1.0	1.2	-	0.95	6.4

Lupus anticoagulant assays (15.05.18)		
Assay	Result	RR
dRVVT screen (ratio)	1.32	0.85 – 1.17
dRVVT confirm (ratio)	1.10	0.90 – 1.10
% correction	16.7	≥ 10
dRVVT screen 50:50 mix (ratio)	1.11	0.90 – 1.07
dRVVT confirm 50:50 mix (ratio)	0.99	0.98 – 1.10
dAPTT screen (ratio)	0.98	0.80 – 1.20

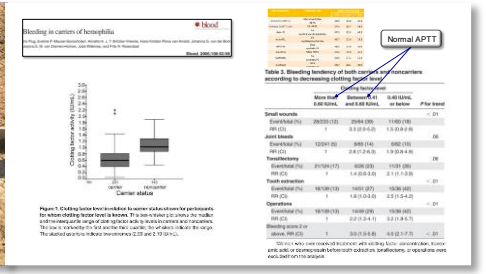
43 year old male
Renal transplant in 2006
Admitted for renal surgery May 2018
No history of bleeding

LA detected by dRVVT but not dilute APTT
Is the LA causing fluctuating elevation of APTT?

FXII deficiency masked by grossly elevated FVIII (± high fibrinogen)

FII:C	(iu/dL)	107	50 – 150
FV:C	(iu/dL)	149	50 – 150
FVII:C	(iu/dL)	83	50 – 150
FX:C	(iu/dL)	125	50 – 150
FVIII:C	(iu/dL)	536	50 – 150
FIX:C	(iu/dL)	111	50 – 150
FXI:C	(iu/dL)	99	58 – 120
FXII:C	(u/dL)	27	50 – 150

FXII deficiency masked by elevated FVIII



Haemophilia A carriers with bleeding tendency, FVIII 40-60%, normal APTT

9 year old boy
Non-consanguineous parents
Mild bleeding tendency: easy bruising, occasional epistaxis, bled excessively after tonsillectomy
some paternal relatives, including father, had mild bleeding tendency

FVII Padua

Lab 1		
Assay	Result	RR
PT (s)	12	10 – 13
APTT (s)	35	30 - 40
TT (s)	9	9 - 11

Lab 2		
Assay	Result	RR
PT (s)	30	12 – 15
PT 50:50 mix (s)	14	12 - 15
APTT (s)	37	32 – 42
TT (s)	11	10 - 12
FVII:C (iu/dL)	7	50 – 150

FVII deficiency that cannot manifest in every PT

Ever wondered what a normal PT or APTT really tell you


Thank you for your attention