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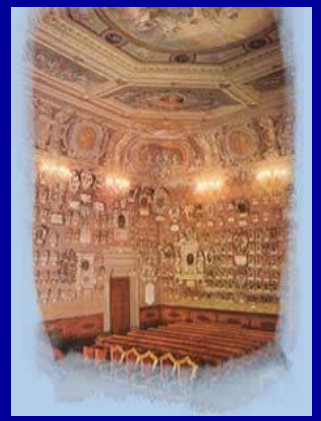

MASTER DI II° LIVELLO IN
MEDICINA VASCOLARE E MALATTIE TROMBOTICO-EMORRAGICHE

EZIOPATOGENESI DELLA TROMBOSI

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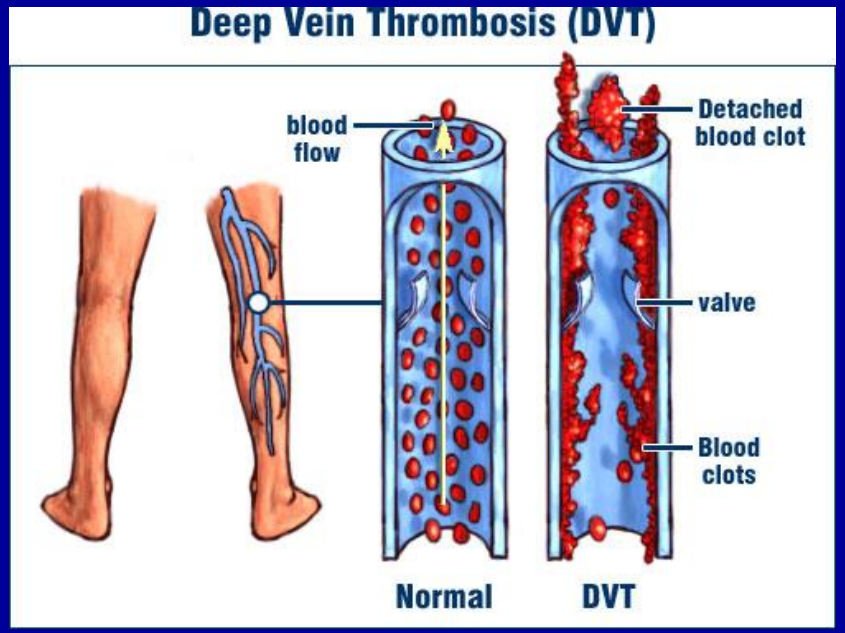
UOC Clinica Medica 1
ad indirizzo Emostasi e Trombosi ed Osservazione Rapida Intensiva
Azienda Ospedale Università di Padova

Venous Thromboembolism (VTE)

- ✓ Venous thrombosis is the process of clot formation within veins.
- ✓ Clot formation occurs predominantly in the vessels of the legs, giving rise to deep venous thrombosis (DVT), or in the lung, resulting in pulmonary embolism (EP).



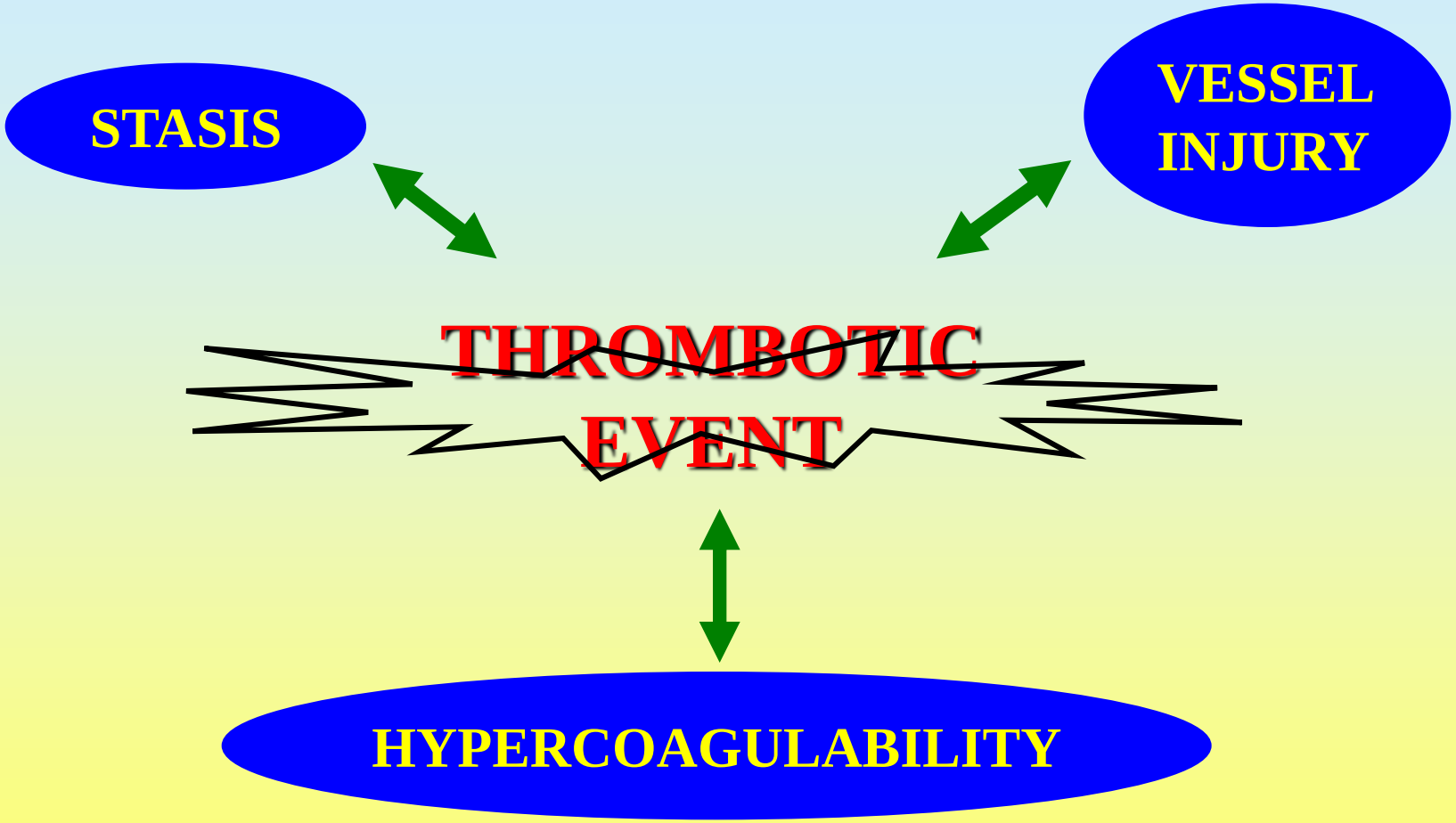
Epidemiology

- ✓ VTE occurs for the first time in 1-2 per 1000 person-years.
- ✓ VTE has a high prevalence both in the community and in the hospitals, and brings a considerable burden of morbidity and possible mortality.
- ✓ Despite anticoagulant therapy, VTE after the initial event has a recurrence rate of ~7% at 6 months.
- ✓ Death occurs in ~ 6% of DVT cases and 12% of PE cases within 1 month of diagnosed.

Pathogenesis of VTE

Virchow's Triad:

1. Alteration in blood flow
2. Vascular endothelial injury
3. Hypercoagulability of blood
   - inherited hypercoagulability
   - acquired hypercoagulability

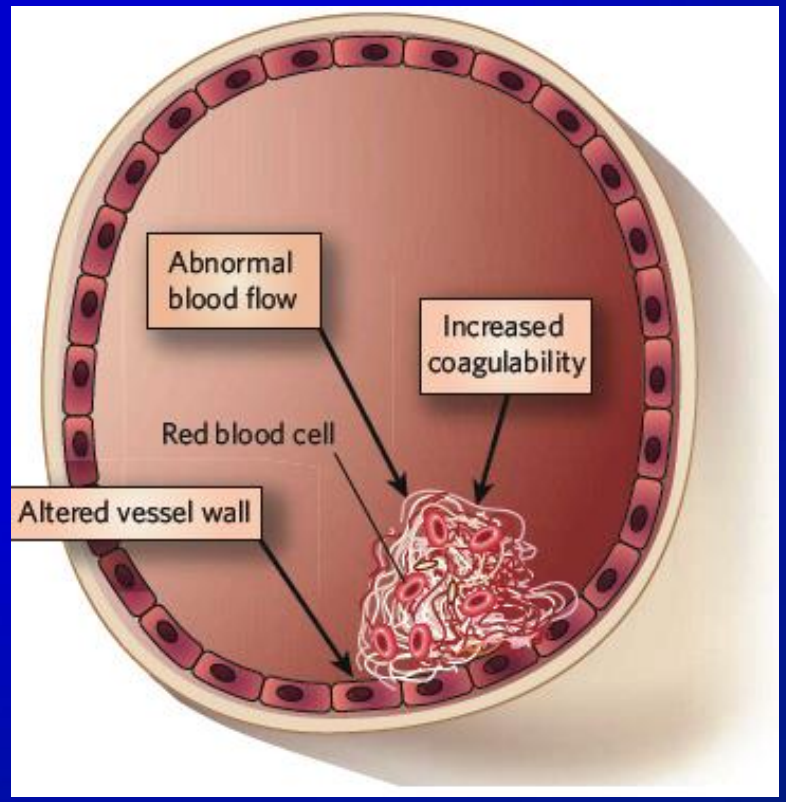

STASIS
VESSEL INJURY
THROMBOTIC EVENT
HYPERCOAGULABILITY

Eziopatogenesi della malattia trombotica

- **Trombosi venosa (trombo rosso)**

- Riduzione del flusso sanguigno

- Ipercoagulabilità



Mackman , 2008

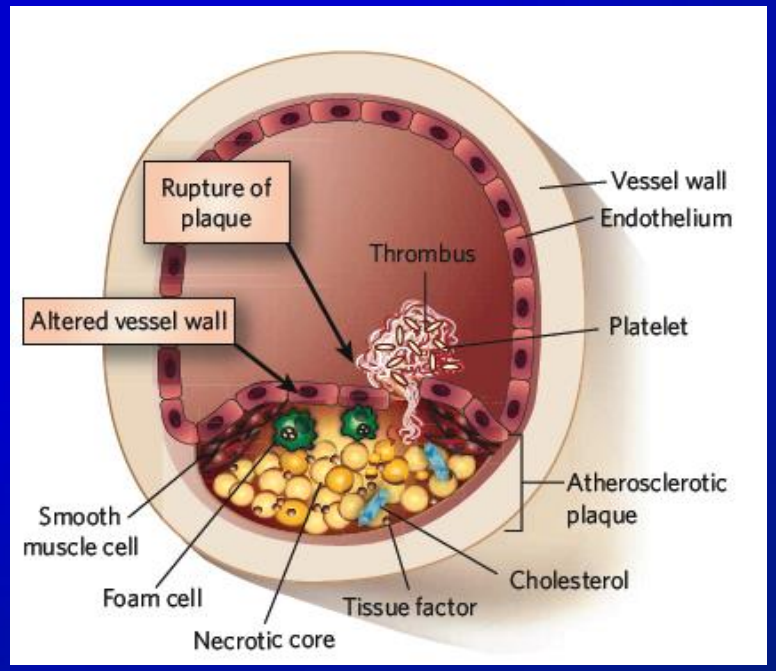
Eziopatogenesi della malattia trombotica

- **Trombosi arteriosa (trombo bianco)**

- Elevato stress del circolo

- Lesione vascolare (rottura placca)

- Alterazioni Piastriniche



Mackman, 2008

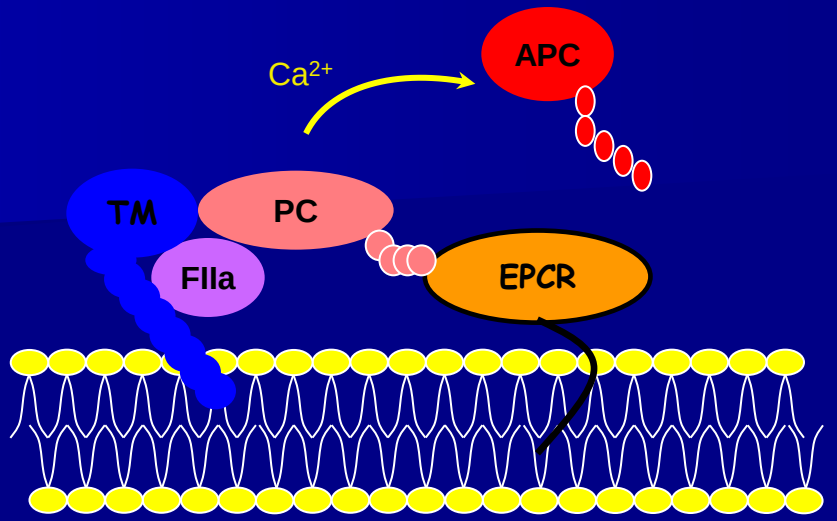
1. Alteration in blood flow

- ✓ bedrest or prolonged immobilization
- ✓ congestive heart or respiratory failure
- ✓ stroke
- ✓ myocardial infarction
- ✓ leg injury
- ✓ lower extremity paralysis
- ✓ extended air travel, “economy class syndrome”

2. Vascular endothelial injury

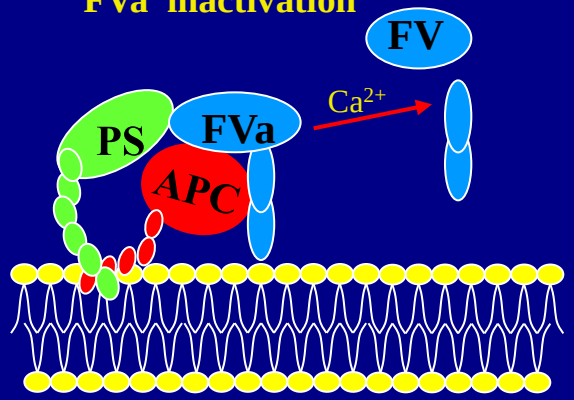
- ✓ trauma
- ✓ recent major surgery
- ✓ malignancy
- ✓ presence of a central venous catheter
- ✓ hIstory of DVT
- ✓ varicose veins
- ✓ chemotherapy
- ✓ intravenous drug use

PROTEIN C ANTICOAGULANT PATHWAY

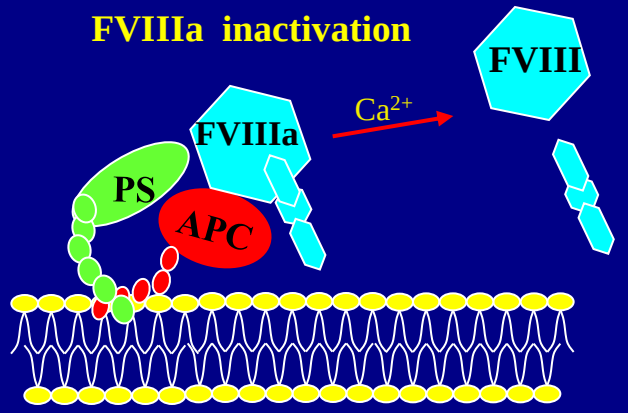


EPCR together with Thrombin-TM complex promotes PC activation (APC)

FVa inactivation



FVIIIa inactivation



3. Inherited hypercoagulability

- ✓ defects of Antithrombin
- ✓ defects of Protein S
- ✓ defects of Protein C
- ✓ APC resistance (Factor V Leiden)
- ✓ G20210A Prothrombin gene mutation
- ✓ Other prothrombin mutations
- ✓ dysfibrinogenemia
- ✓ increased levels of factor VIII, IX, XI

3. Aquired hypercoagulability

- ✓ antiphospholipid syndrome (APS)
- ✓ hyperhomocysteinemia
- ✓ malignancy
- ✓ obesity
- ✓ prolonged bed stays, especially in the elderly
- ✓ myeloproliferative disorders
- ✓ paroxysmal nocturnal hemoglobinuria (PNH)

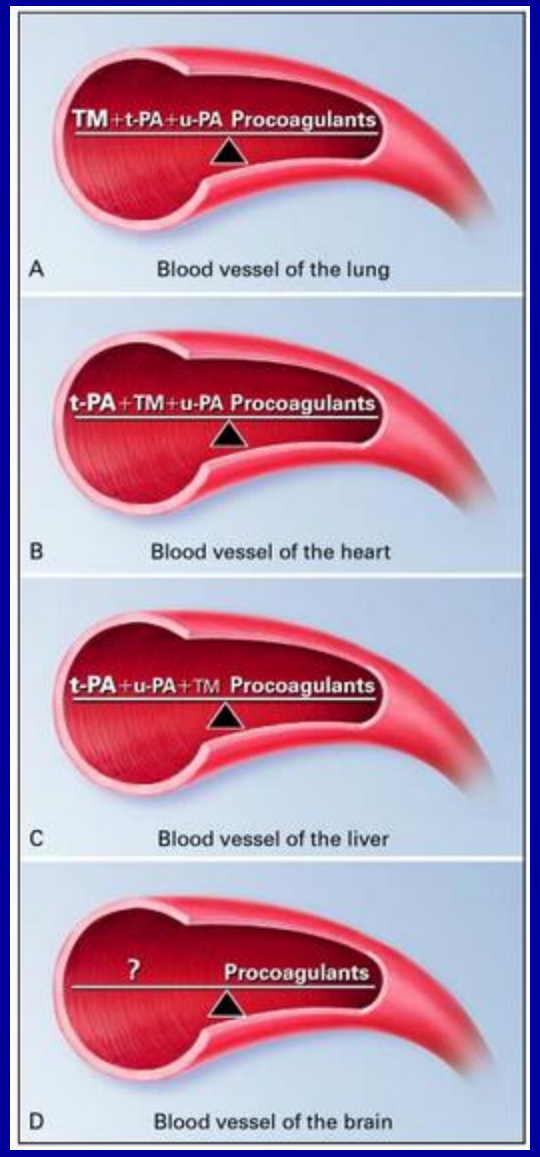
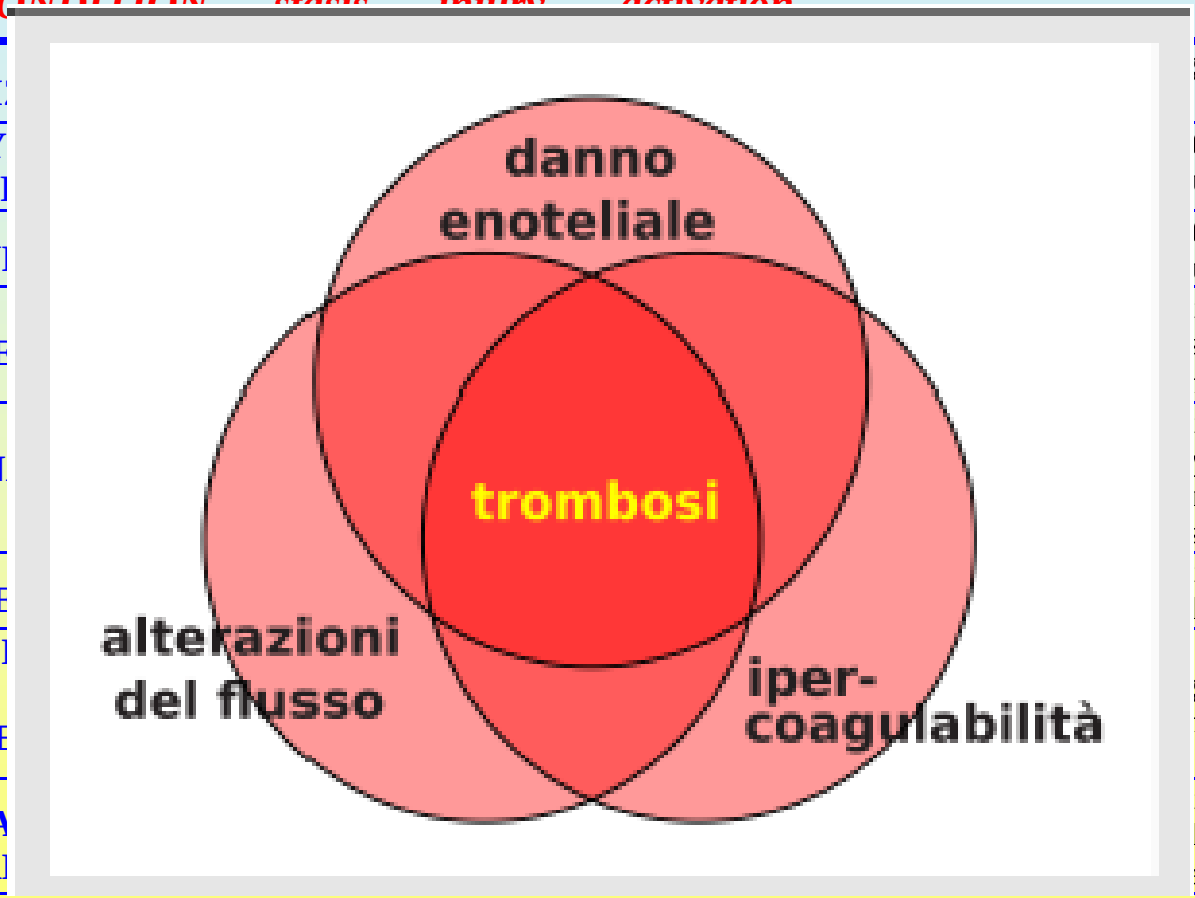


Figure 2. Vascular-Bed–Specific Hemostasis.

The interaction of the various anticoagulant and procoagulant forces promotes overall hemostasis, but the actual components of this interaction differ from one vascular bed and one organ to another. Thrombomodulin (TM) is more important in maintaining the hemostatic balance of the lungs and heart (Panels A and B) than it is in the liver, whereas the fibrinolytic pathway (tissue-type plasminogen activator [t-PA] and urokinase-type plasminogen activator [u-PA]) is important in mediating blood fluidity in all three vascular beds (Panels A, B, and C). Neither thrombomodulin nor fibrinolysis is essential in maintaining balanced hemostasis in the blood vessels of the brain (Panel D). The physiologically relevant natural anticoagulant mechanisms that are operative in this vascular bed have not been identified.

Rosenberg RD 1999

Potential thromboembolic mechanism in the hypercoagulable states (acquired conditions)



RISK FACTOR / CLINICAL CONDITION	Venous stasis	Vascular injury	Haemostatic activation	COMMENTS
IMMOBILI				loss of flow
SURGERY OPERATIVI				tion ury
TRAU				tion ury
ACVANCE				y sease neration
MALIGN				y y tumor nts and
ADVANCE				y low
PRIOR CHRONIC VARICOSE				nage
PREGNA POST PA				y low els
ORAL CONTRACEPTIVE TREATMENT	–	–	++	↓ PS and AT levels
LUPUS ANTICOAGULANT / APA SYNDROME	–	+	++	Vascular injury-vasculitis Impaired fibrinolysis Impaired PC activation

Pathogenesis of VTE:
evidence of multiple interrelated cases

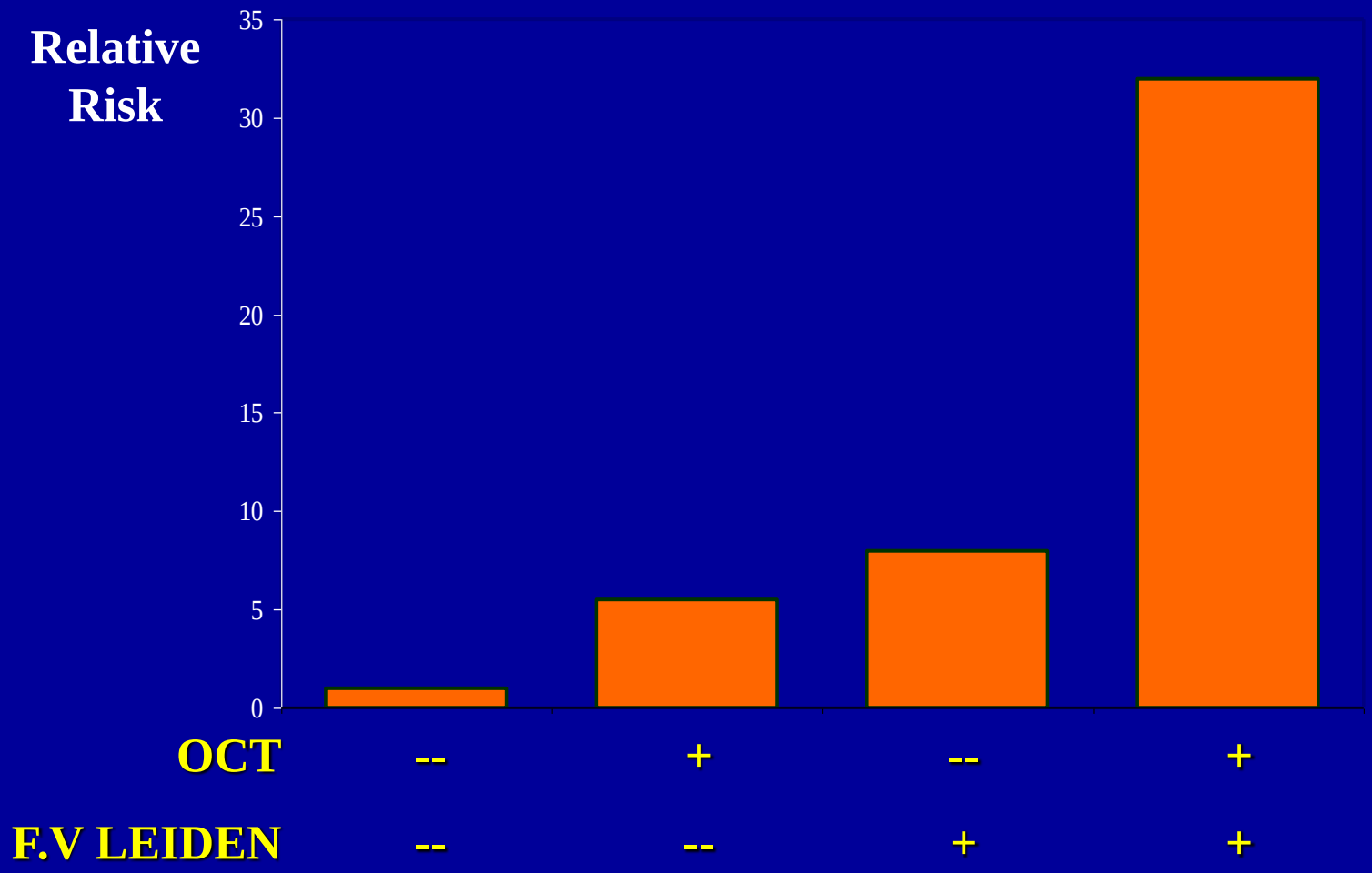
- ✓ VTE is the result of interactions among multiple genetic and enviromental risk factors.
- ✓ Individual risk factors, or combinations of them, can have important implications for the type and duration of appropriate prophylaxis.
- ✓ About 50% of DVT are still defined as “idiopathic”.

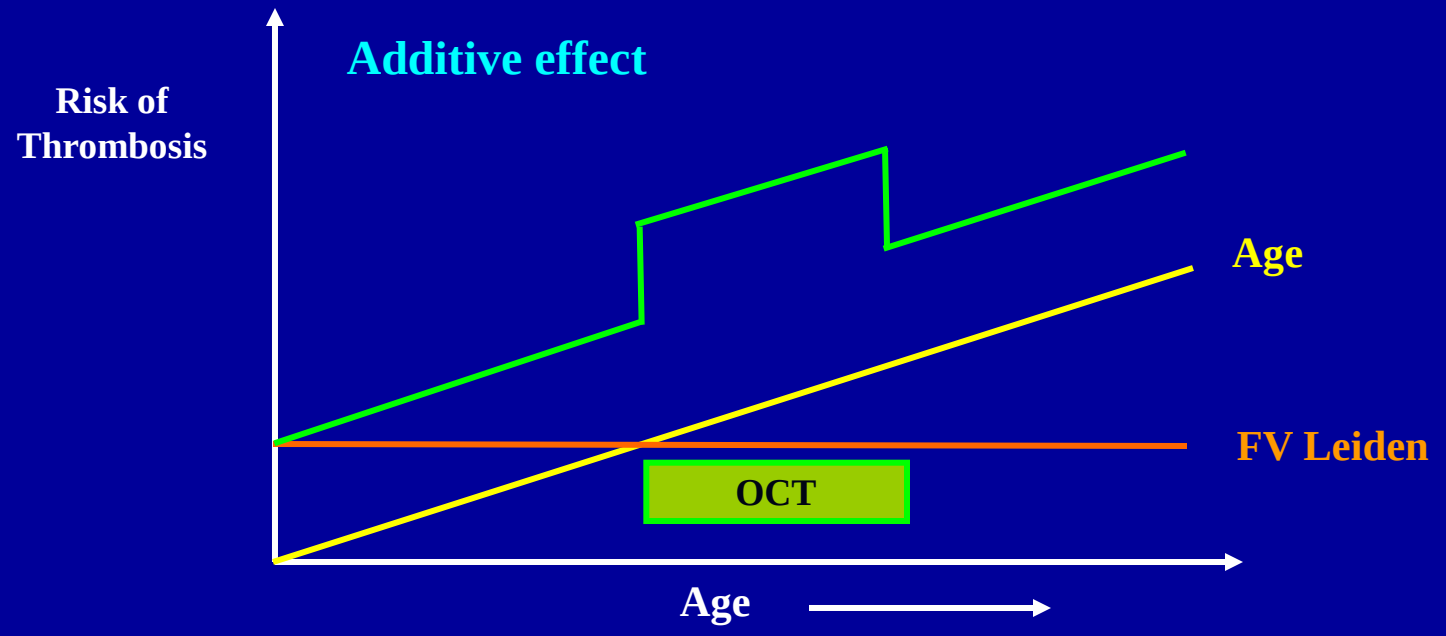
Annual incidence of VTE according to age

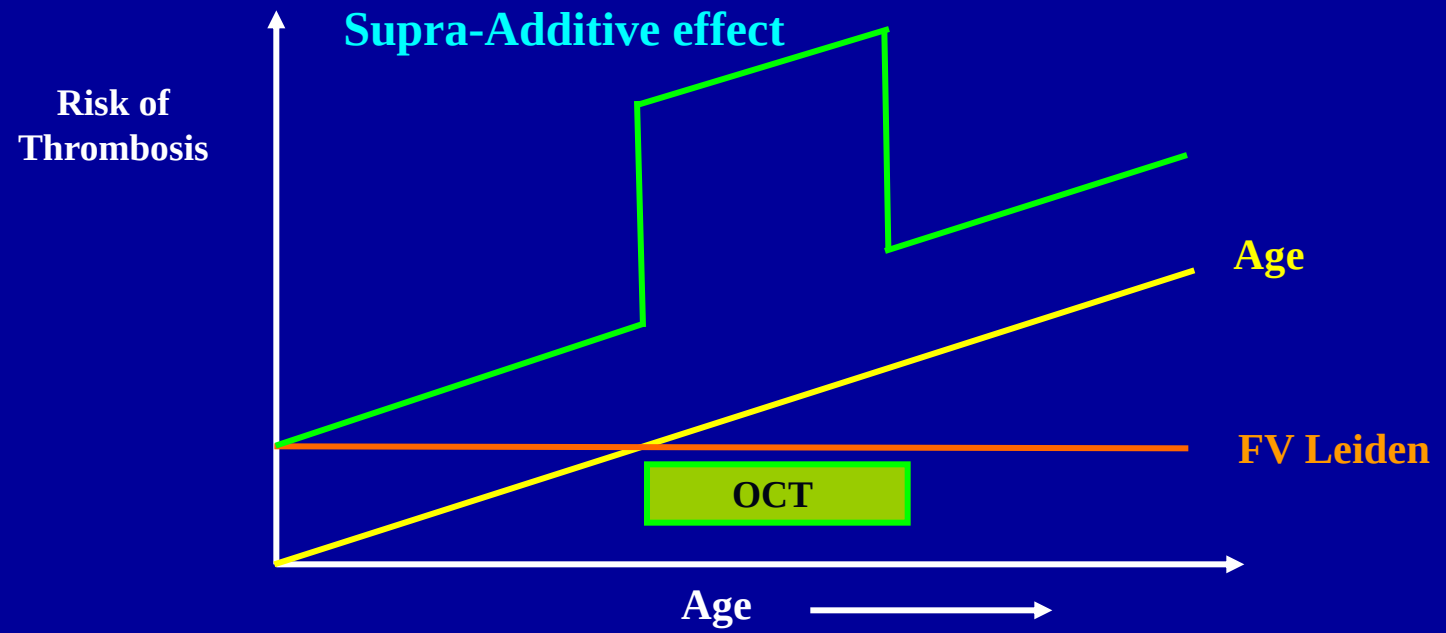
- < 20 years 1:100000 persons
- 20 -40 years 1:10000 persons
- 41-75 years 1:1000 persons
- >75 years 1:100 persons

Interrelation F.V Leiden / OCT

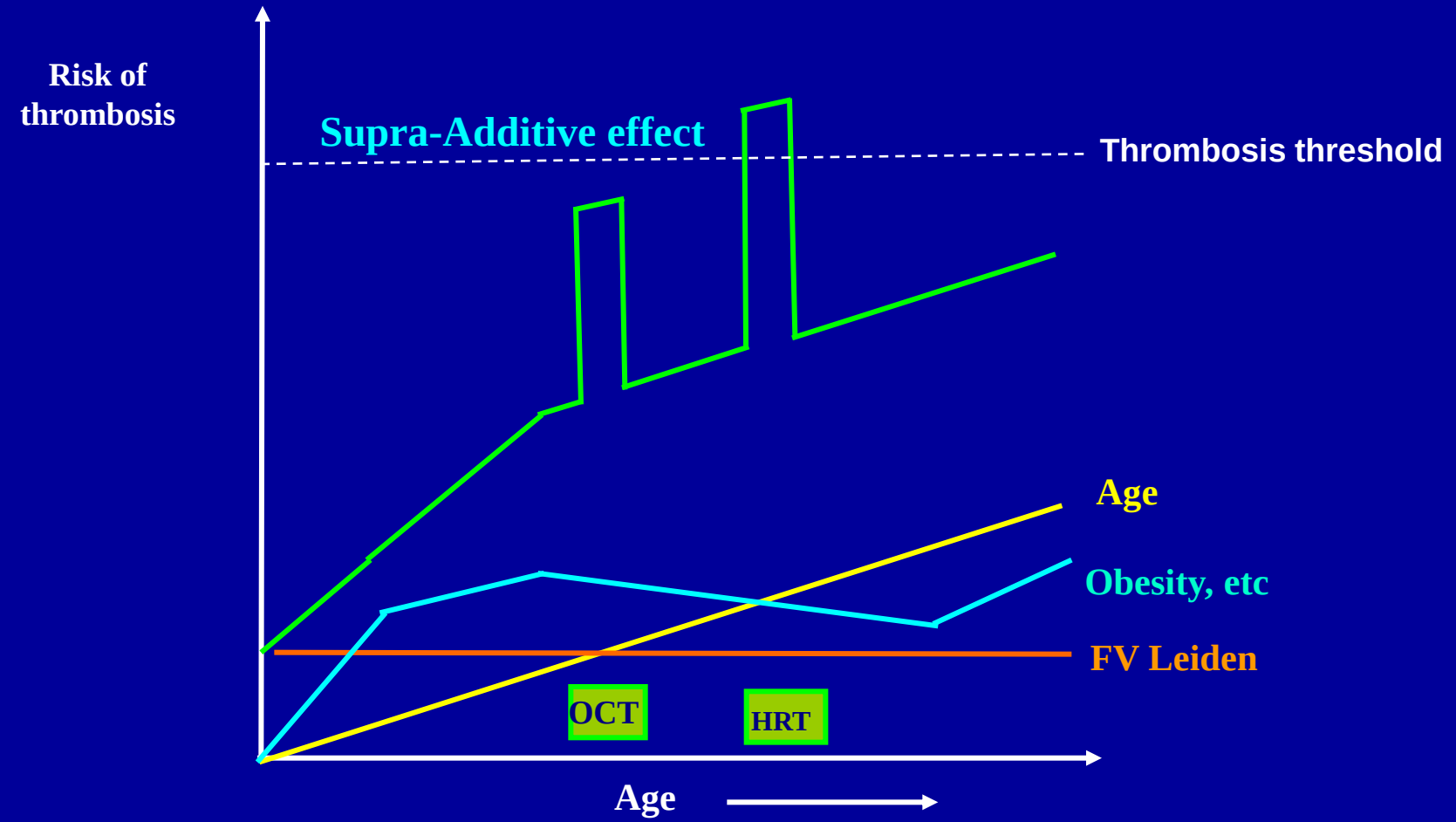
(Vandenbroucke et al, *Lancet 1996)*




Additive effect
Risk of Thrombosis
Age
FV Leiden
OCT
Age


Supra-Additive effect
Risk of Thrombosis
Age
FV Leiden
OCT
Age

A MULTIFACTORIAL MODEL FOR THROMBOSIS IN YOUNG WOMEN WITH THROMBOPHILIA ON HORMONAL THERAPY



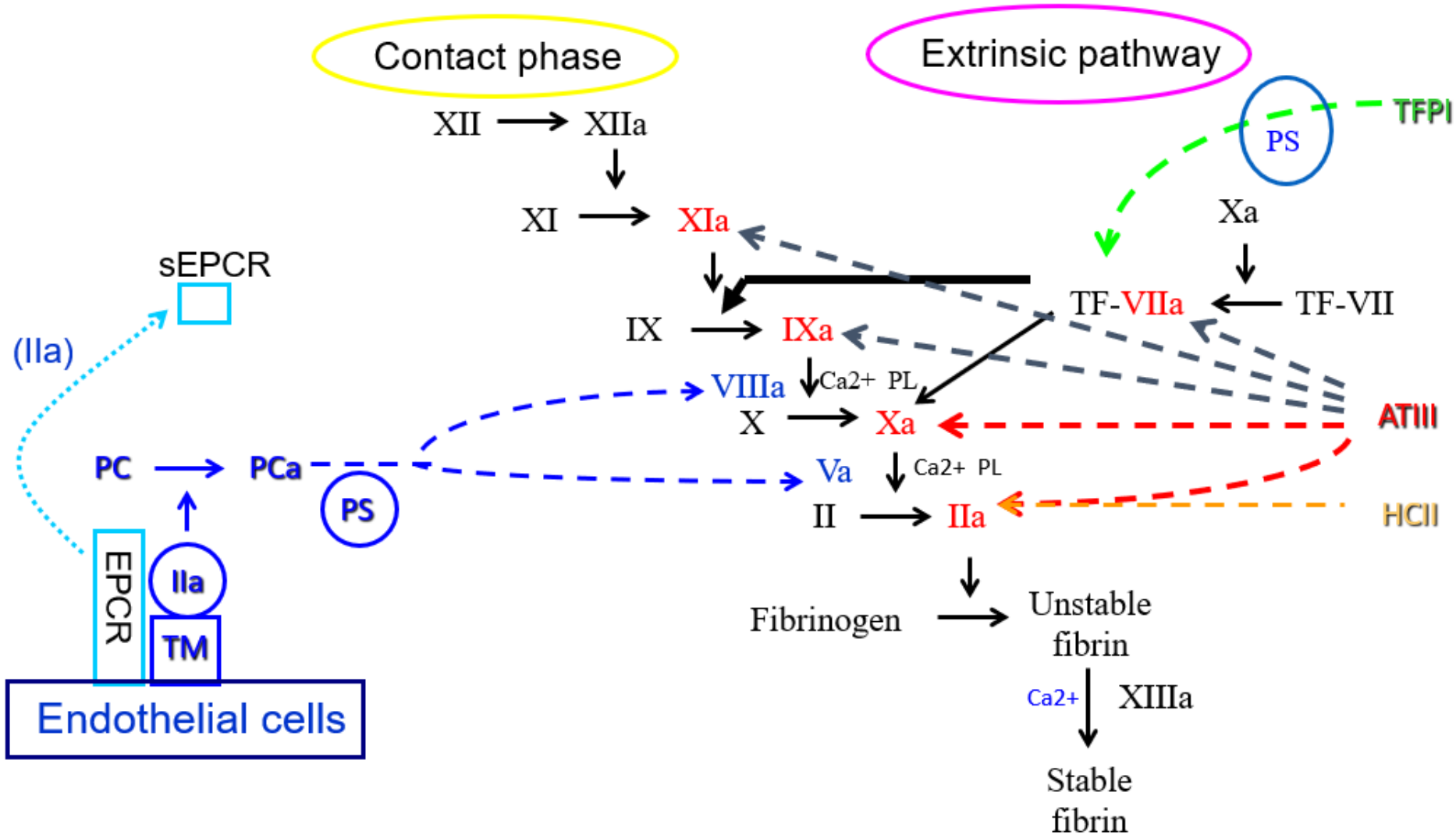
THROMBOPHILIA

A clinical condition characterised by increased tendency to venous thrombosis which may develop spontaneously and at young age and which cannot be satisfactorily explained by acquired risk factors.

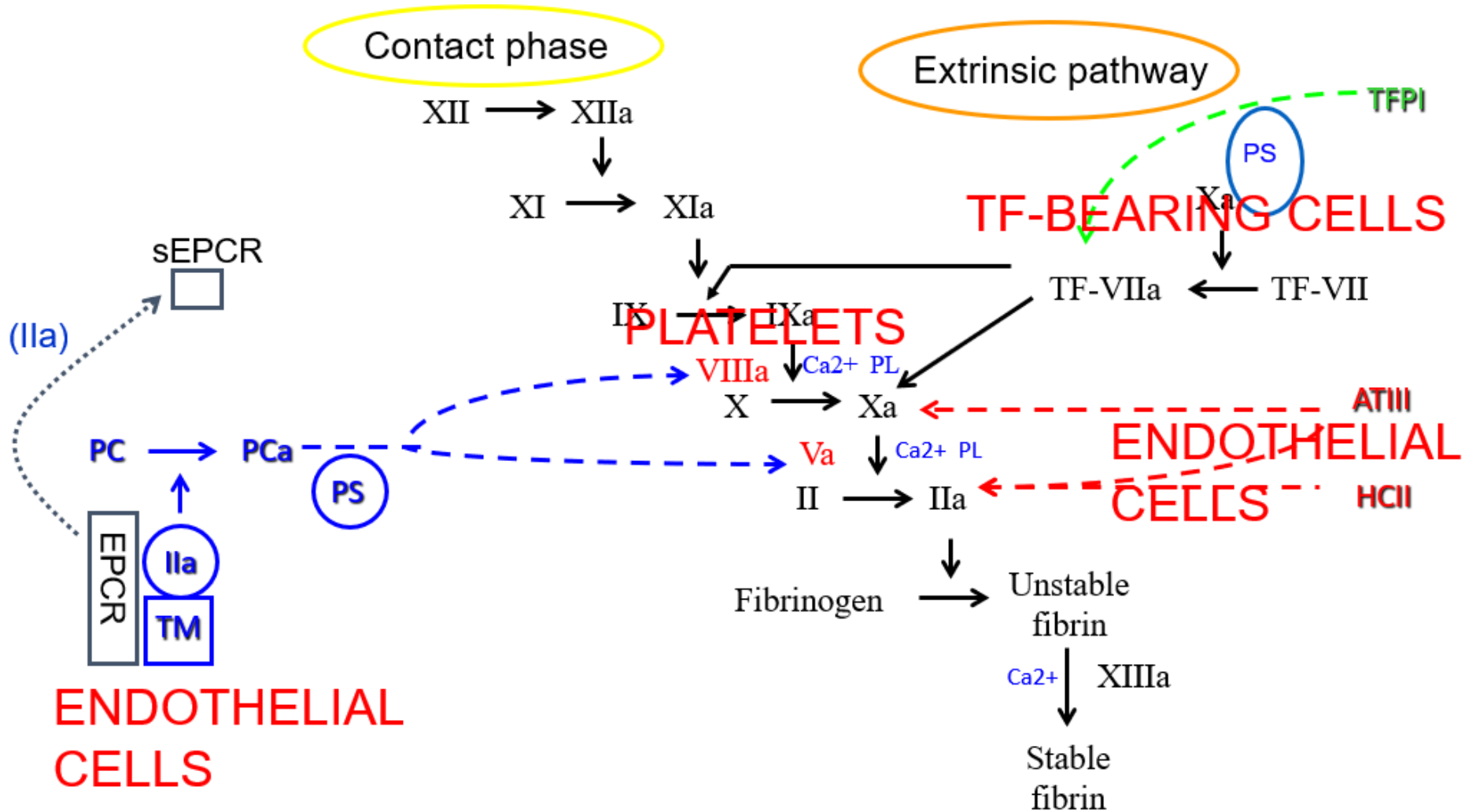
CLINICAL MANIFESTATIONS OF THROMBOPHILIA

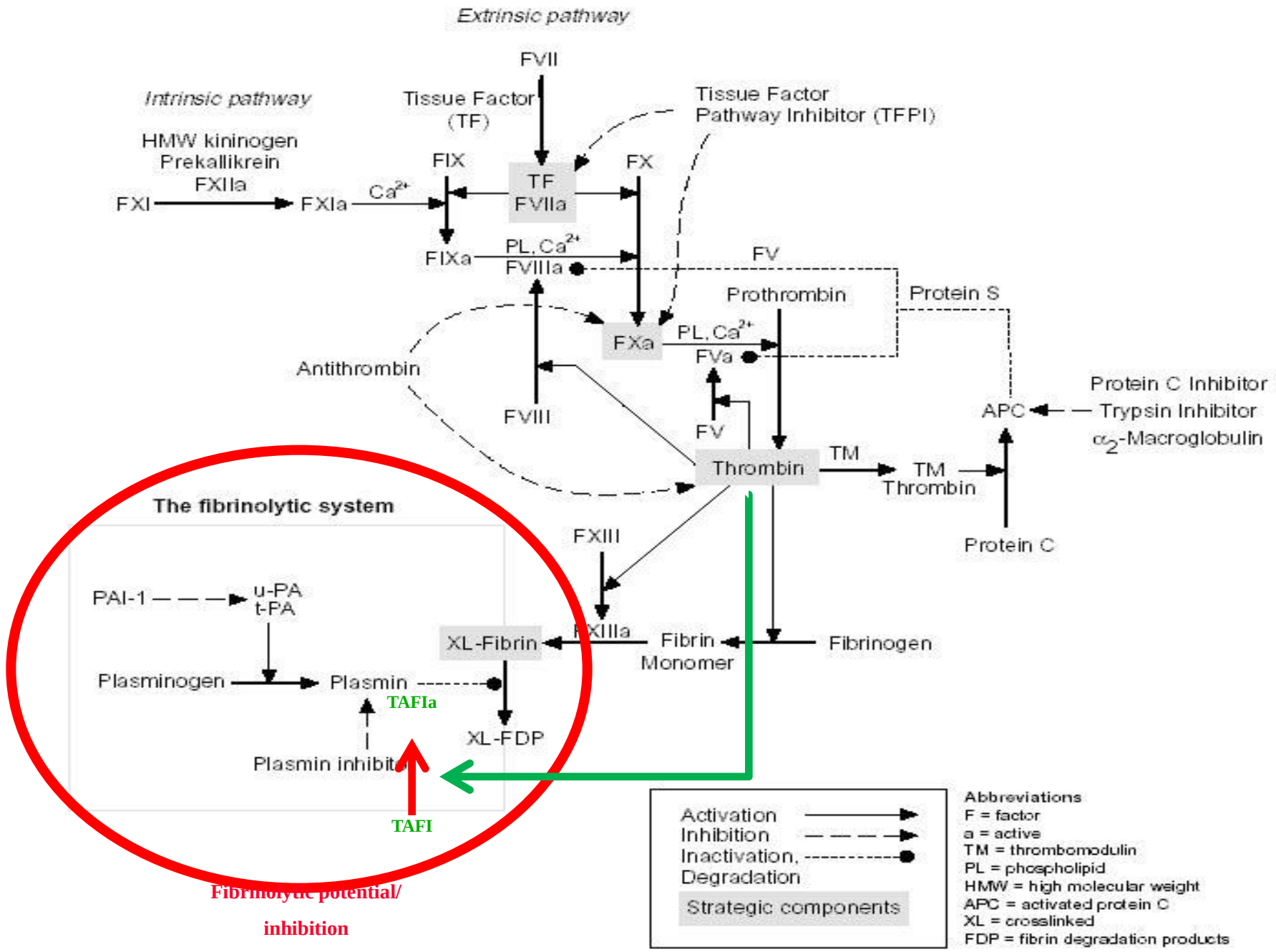
1. **Family history of venous thromboembolism involving males and females**
2. **Spontaneous or risk period related venous thromboembolism at a young age (<45 years)**
3. **Recurrent venous thromboembolism**
4. **Thrombosis in unusual site (cerebral sinuses, mesenterial, portal)**
5. **Recurrent foetal loss, Preeclampsia, HELLP syndrome**
6. **Vitamin K antagonist-induced skin necrosis**
7. **Neonatal purpure fulminans**
8. **Heparin resistance**

CLOTTING CASCADE AND SYSTEMS OF PHYSIOLOGICAL INHIBITION

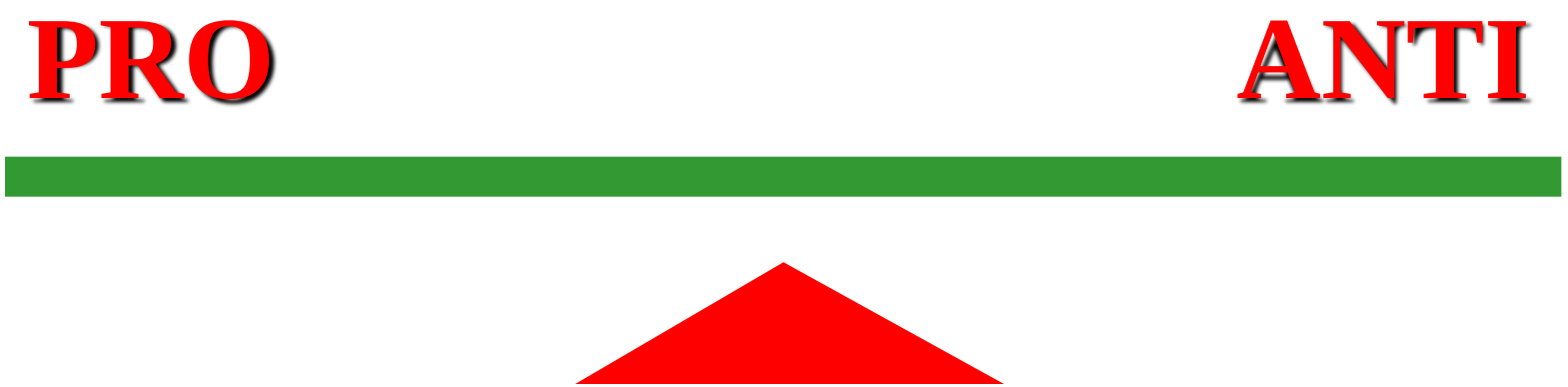


CLOTTING CASCADE AND SYSTEMS OF PHYSIOLOGICAL INHIBITION




Extrinsic pathway
FVII
Intrinsic pathway
Tissue Factor (TF)
Tissue Factor Pathway Inhibitor (TFPI)
HMW kininogen
Prekallikrein
FXIIa
FXI
FXIa
Ca2+
FIX
TF FVIIa
FX
FIXa
PL, Ca2+
FVIIIa
FV
Prothrombin
Protein S
FXa
PL, Ca2+
FVa
Antithrombin
FVIII
FV
Protein C Inhibitor
Trypsin Inhibitor
α2-Macroglobulin
APC
Thrombin
TM
TM Thrombin
Protein C
FXIII
FXIIIa
XL-Fibrin
Fibrin Monomer
Fibrinogen
PAI-1
u-PA
t-PA
Plasminogen
Plasmin
TAFIa
XL-FDP
Plasmin inhibitor
TAFI
The fibrinolytic system
Fibrinolytic potential/ inhibition
Activation
Inhibition
Inactivation, Degradation
Strategic components
Abbreviations
F = factor
a = active
TM = thrombomodulin
PL = phospholipid
HMW = high molecular weight
APC = activated protein C
XL = crosslinked
FDP = fibrin degradation products

THE HEMOSTATIC / THROMBOTIC BALANCE



Thrombophilia

Inherited and acquired thrombophilic conditions

Inherited	Acquired
Antithrombin deficiency	Pregnancy
Protein C deficiency	Immobility/Surgery
Protein S deficiency	Trauma
Factor V Leiden	Hormonal therapy
Prothrombin 20210 mutation	Antiphospholipid syndrome
	Malignancy
Increased levels of coagulation factors (Factor IX Padua)	Myeloprolipferative disorders
	HIT
Prothrombin defects (AT resistance)	PNH
	Behcet's disease
Disorders of plasmin generation	Nephrotic syndrome
Dysfibrinogenemia	
Hyperhomocysteinemia	
Blood group	
UNKNOWN	

Thrombophilia and risk of thrombosis

Table 1. Increased thrombotic risk in hereditary and acquired thrombophilia

Thrombophilia	Relative risk for a first VTE (compared to community controls)
Hereditary thrombophilia	
Factor V Leiden	
Heterozygous	3-7x
Homozygous	80x
Prothrombin G20210A	
Heterozygous	2-3x
Homozygous	5x
Double heterozygosity (FVL and prothrombin G20210A)	6x
AT deficiency	5x
Protein C deficiency	4-6.5x
Protein S deficiency	1-3x
Pseudohomozygous FVL	80x
Factor IX Padua	10x
AT resistance	2-3x
Non-0 blood type	2x
Factor VIII ≥150 IU/dL	3-5x
Factor IX ≥129 IU/dL	2.3x
Factor XI ≥121 IU/dL	2x
Acquired thrombophilia	
Antiphospholipid antibody syndrome	3-10x
Hyperhomocysteinaemia	1.5-3x

Campello E. et al. Expert Rev Hematol 2019

Thrombophilia

Thrombophilia is a generic term used for several acquired or hereditary conditions that indicates a patient has a higher-than-normal risk of VTE. The heritability for VTE, i.e. the proportion of variance attributable to genetic effects, is estimated to be as high as 60%. There are several known genetically determined defects associated with thrombophilia, collectively linked to at least a third of cases of VTE.

TROMBOFILIA lieve

- ✓ Fattore V Leiden *eterozigote*
- ✓ Variante protrombinica G20210A *eterozigote*

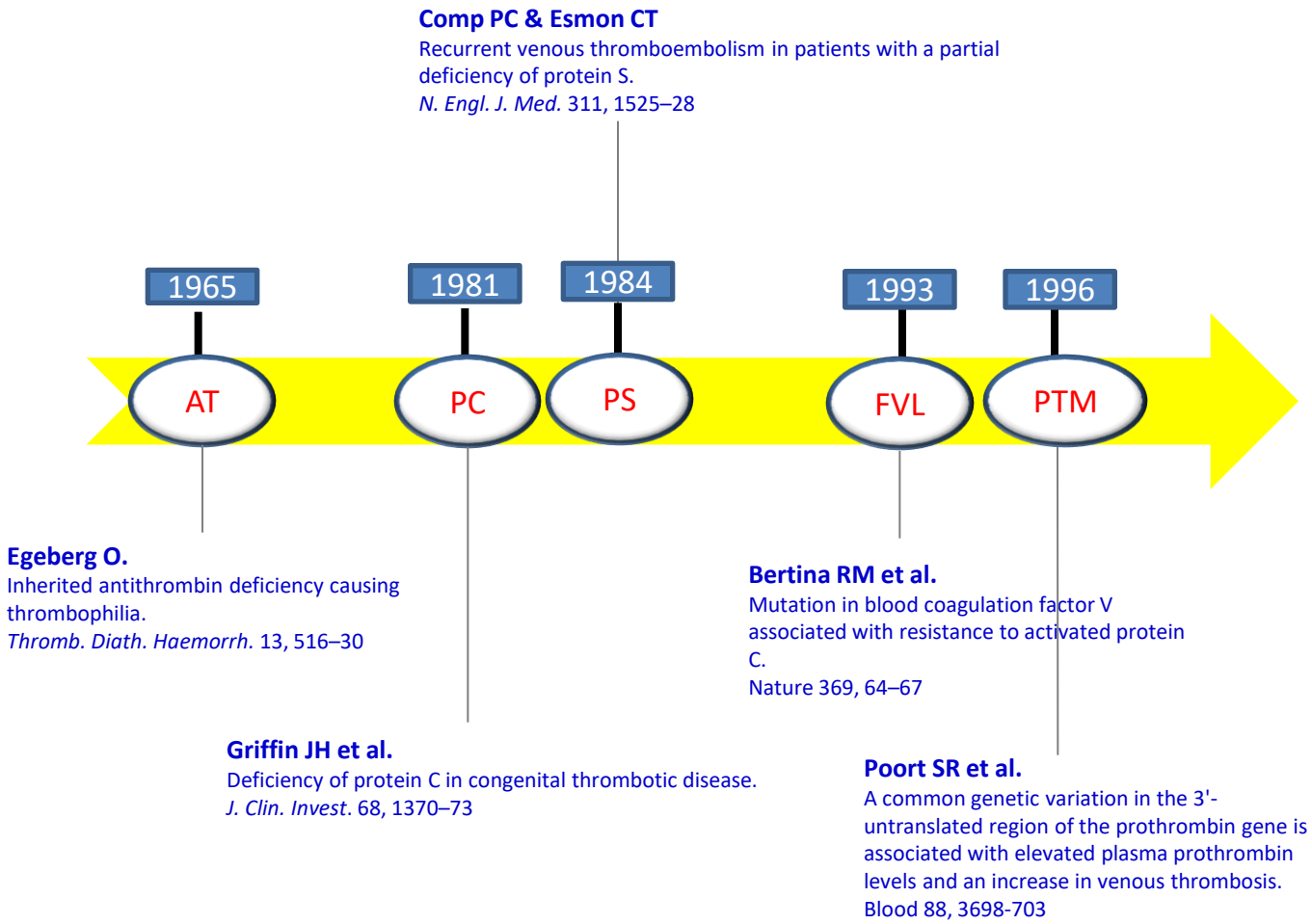
TROMBOFILIA severa

- ✓ Difetto di Antitrombina
- ✓ Difetto di Proteina C
- ✓ Difetto di Proteina S
- ✓ Fattore V Leiden *omozigote*
- ✓ Variante protrombinica G20210A *omozigote*
- ✓ Difetti combinati

TROMBOFILIA acquisita

- ✓ APS

Middeldorp S, et al. Blood Adv 2023



Comp PC & Esmon CT
Recurrent venous thromboembolism in patients with a partial deficiency of protein S.
N. Engl. J. Med. 311, 1525–28

Egeberg O.
Inherited antithrombin deficiency causing thrombophilia.
Thromb. Diath. Haemorrh. 13, 516–30

Griffin JH et al.
Deficiency of protein C in congenital thrombotic disease.
J. Clin. Invest. 68, 1370–73

Bertina RM et al.
Mutation in blood coagulation factor V associated with resistance to activated protein C.
Nature 369, 64–67

Poort SR et al.
A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis.
Blood 88, 3698-703

Prevalence of inherited thrombophilia and RR estimates for major clinical manifestations

	AT deficiency	PC deficiency	PS deficiency	FVL	PT 20210 mutation
Prevalence in general population	**0.02%**	**0.2%**	**0.03-0.13%**	**3-7%**	**0.7-4%**
Prevalence in consecutive VTE	**1%**	**3%**	**2%**	**20%**	**5%**
RR for first VTE	**5-10**	**4-6.5**	**1-10**	**3-5**	**2-3**
RR for recurrent VTE	**1.9-2.6**	**1.4-1.8**	**1.0-1.4**	**1.4**	**1.4**
RR for arterial thrombosis	**-**	**-**	**-**	**1.3**	**0.9**
RR for pregnancy complications	**1.3-3.6**	**1.3-3.6**	**1.3-3.6**	**1.0-2.6**	**0.9-1.3**

Adapted from Middeldorp, Hematology 2016

Estimated incidence of a first episode of VTE

	AT, PC or PS deficiency	FVL hetero	PT 20210 mutation	FVL homo
Overall, %/y	**1.5 (0.7-2.8)**	**0.5 (0.1-1.3)**	**0.4 (0.1-1.1)**	**1.8 (0.1-4)**
Surgery, trauma or immobilization, %/episode	**8.1 (4.5-13.2)**	**1.8 (0.7-4.0)**	**1.6 (0.5-3.8)**	**-**
Pregnancy, %/pregnancy	**4.1 (1.7-8.3)**	**2.1 (0.7-4.9)**	**2.3 (0.8-5.3)**	**16.3**
During pregnancy, %	**1.2 (0.3-4.2)**	**0.4 (0.1-2.4)**	**0.5 (0.1-2.6)**	**7.0**
Postpartum, %	**3.0 (1.3-6.7)**	**1.7 (0.7-4.3)**	**1.9 (0.7-4.7)**	**9.3**
OCT use, %/y of use	**4.3 (1.4-9.7)**	**0.5 (0.1-1.4)**	**0.2 (0-0.9)**	**-**

Adapted from Middeldorp, Hematology 2016

Testing for thrombophilia

WHAT?
Routine tests for screening of thrombophilia

Antithrombin	functional (chromogenic)
Protein C	functional (chromogenic or clotting)
Protein S	[functional (clotting)] or free antigen
APC Resistance	dilution with FV deficient plasma

Factor V Leiden	**PCR**
Prothrombin G20210A	**PCR**

LAC	diluted aPTT + dRVVT (+ → go on)
Antiphospholipid abs	anticardiolipin or anti beta2-GPI

Homocysteine	HPLC or ELISA or FPIA

Thrombophilia screening in Padua

Esame	*Esito*	*Unità*	*Valori di riferimento*
Fattori della coagulazione			
Fattore II attività	101.7	%	*80.0 - 120.0*
Fattore VIII attività *(Metodo: aPTT one stage)*	94.8	%	*60.0 - 160.0*
Fattore IX attività *(Metodo: aPTT one stage)*	93.8	%	*80.0 - 120.0*
Fattore X attività	87.8	%	*80.0 - 120.0*
Fattore XI attività	97.1	%	*80.0 - 120.0*
Inibitori della coaugulazione			
Antitrombina attività cromogenica	103.0	%	*80 - 120*
Proteina C attività coagulometrica	***75.4***	%	*80 - 120*
Proteina C attività cromogenica	111.2	%	*70 - 130*
Proteina C antigene (ELISA)	115	%	*80 - 120*
Proteina S attività coagulometrica	85.4	%	*70 - 130*
Proteina S antigene libera (ELISA)	83	%	*80 - 120*
Proteina S antigene totale (ELISA)	117	%	*80 - 120*

Thrombophilia screening in Padua

Resistenza alla Proteina C attivata

APC sensitivity ratio	***1.89***	*>2.00*
APC sensitivity ratio normalization	***0.63***	*>0.84*

Analisi dei Polimorfismi (trombofilici)

Mutazione fattore V Leiden	***Presente Eterozigote***
Variante protrombinica G20210A	Assente

Fibrinolisi

Plasminogeno attività	100.5	*%*	*75.0 - 140.0*
PAI antigene	7.7	*ng/ml*	*1.0 - 25.0*

Anticorpi antifosfolipidi

Anticorpi anti-beta2-glicoproteina I IgG	0.9	*U/ml*	*<8*
Anticorpi anti-beta2-glicoproteina I IgM	0.7	*U/ml*	*<8*
Anticorpi anti cardiolipina IgG	0.8	*U/ml*	*<10*
Anticorpi anti cardiolipina IgM	1.8	*U/ml*	*<10*
dRVVT	36.4	*sec*	*26.0 - 45.0*
aPTT-LA	36.2	*sec*	*32.0 - 43.0*

Thrombophilia screening in Padua

Commento: ***Resistenza alla proteina C attivata da presenza di fattore V Leiden a livello eterozigote.***
Lieve riduzione "spuria" dei livelli di proteina C coagulometrica.
Altri parametri esplorati nella norma o ai limiti di norma.
Si consiglia studio dei familiari disponibili per fattore V Leiden, ove non già eseguito.

Antithrombin deficiency

- First described by Egeberg in a Swedish family with recurrent VTE (1965)
- Autosomic dominant, prevalece 1/500-1/5000
- Type I → quantitative defect
- Type II → qualitative (most frequent but less associated with thrombosis)
- A large population-based study showed that AT deficiency was associated with a five-fold (95% CI 0.7– 34) increased risk of a first episode of deep venous thrombosis

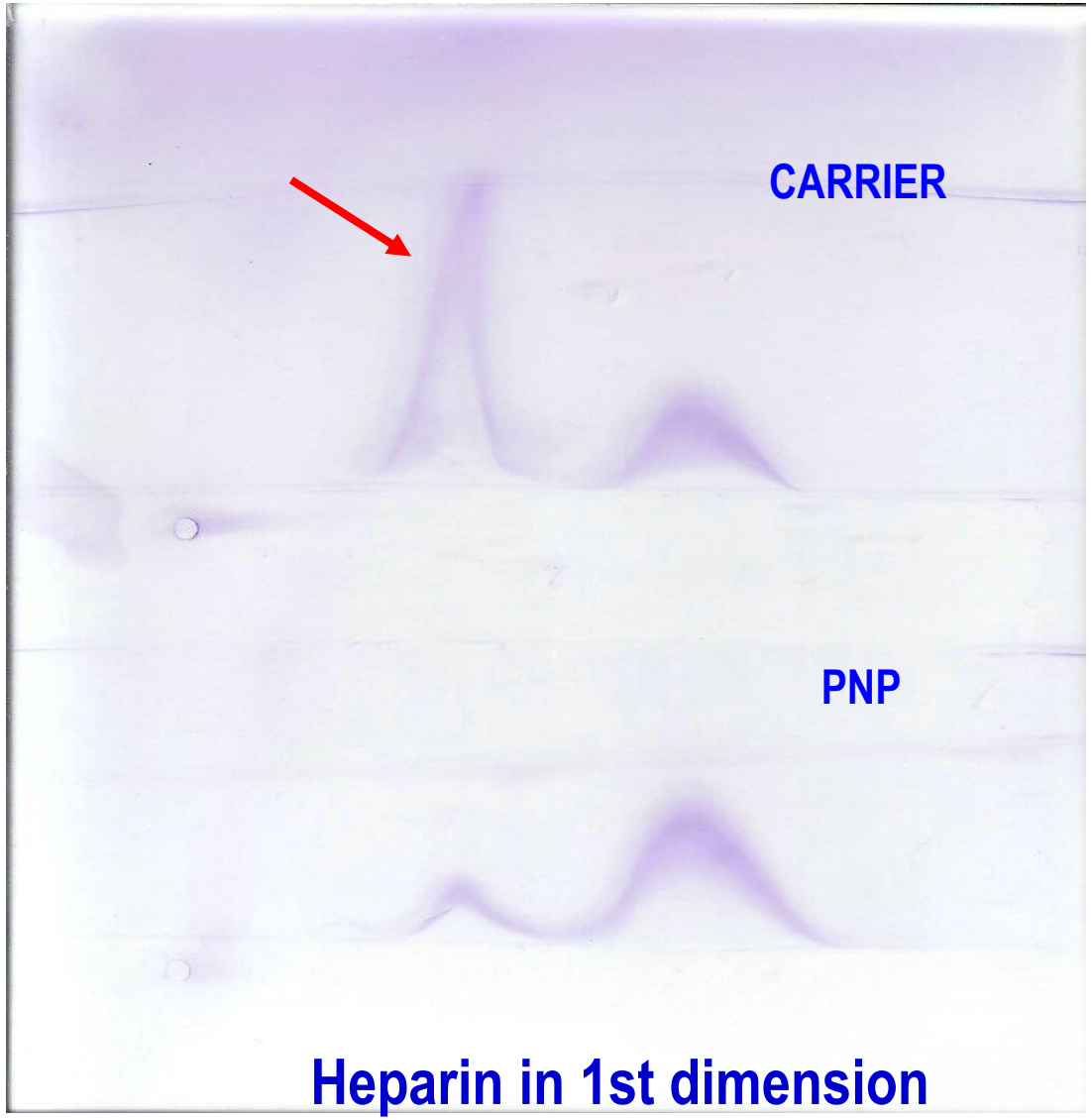
Patnaik e Moll, Haemophilia, 2008
Middeldorp, J Thromb Thrombolysys, 2011

Frequency of antithrombin defects in the general population

- CLINICALLY SYMPTOMATIC FAMILIAL AT DEFICIENCY 1/5000 -1/2000

-ASYMPTOMATIC TYPE IIb DEFECTS (HEPARIN BINDING SITE) 1/350

Type IIb Antithrombin defect (so called heparin binding defect)



Acquired causes of antithrombin deficiency

- Acute thrombotic event
- DIC and sepsis
- Surgery
- Hepatopathies
- Nephrotic syndrome
- Treatment for ALL with L-asparaginase
- Lack of extended studies !!

Protein C deficiency

- First described by Griffin et al in 1981
- 0,2-0,3% of population as heterozigous defect, 3% of patients with first episode of VTE
- More than 300 mutations
- Three types:
  - I quantitative defect (75% of patients)
  - II qualitative (25% of patients)
  - III combined
- From the clinical point of view, heterozygous PC deficiency is associated with a 4- to 6.5-fold increased risk of VTE, while homozygous deficiency results in severe thrombotic complications in the foetus, neonates or children

Nizzi, Kaplan, Semin Thromb Haemost 1999
Mc Callum, BMJ 2014

TYPE II B PROTEIN C DEFECT (ABNORMAL PC)

	PC antigen (ELISA, Ca^{++} - independent PoAb) % (nv= 80-120)	PC antigen (ELISA, Ca^{++} - dependent MoAb) % (nv= 80-120)	PC activity (amidolytic) % (nv= 75-130)	PC activity (clotting) % (nv= 80-120)
$PC_{R-1L/propeptide}$				
Proposita	102	52	105	53
Sister, normal	105	100	102	105
Sister, affected	100	50	103	50
Supernatant, proposita	15	n.d.	16	n.d.
Supernatant, sister, normal	5	5	<5	<5
Supernatant, sister, affected	17	n.d.	14	n.d.
Subject with PCP2 (PC_{R-1C})				
Plasma	98	48	96	46
Supernatant	38	n.d.	30	n.d.

Simioni et al , *Thromb Haemost 2001*

Acquired C protein deficiencies

- Cirrhosis
- DIC and sepsis
- K vitamin loss
- AVK therapy
- Nephrotic syndrome

Protein S deficiency

- First noted in 1981
- S protein is cofactor of C protein
- Autosomal dominant transmission
- Up to 2,3 % of general population, up to 12% of patients with thrombosis
- Data to be carefully interpreted based on the different lab tests and variability of S protein concentration
- **The real impact on risk for DVT is still debated**

Protein S

	TOTAL AG	FREE AG	ACTIVITY
TYPE I	↓	↓	↓
TYPE II	N	N	↓
TYPE III	N	↓	↓

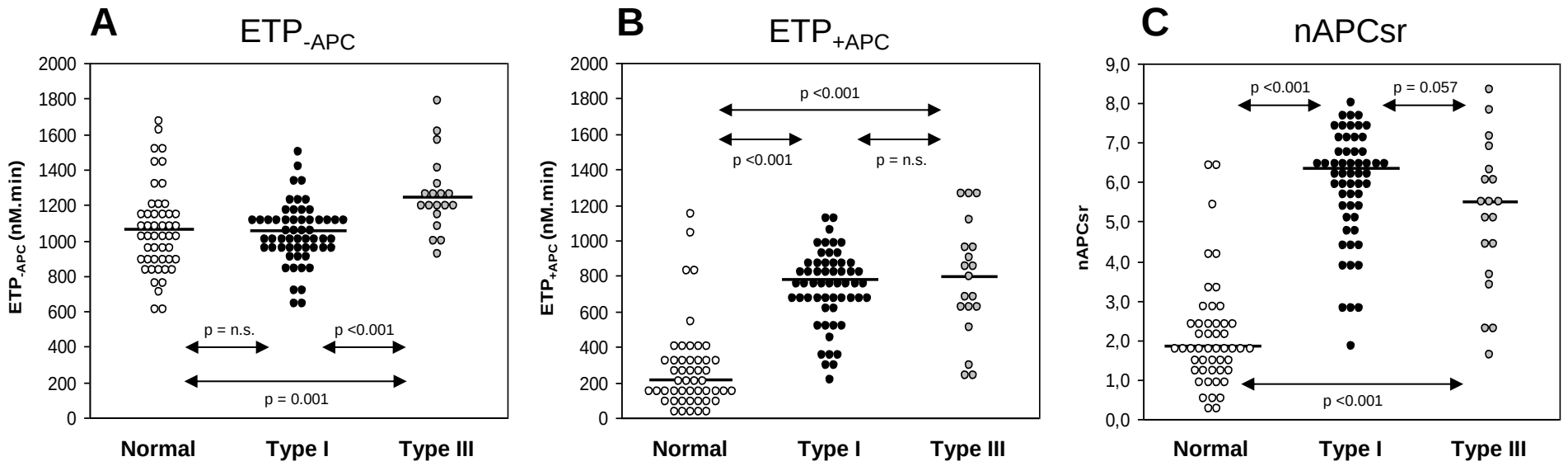
SCREENING TESTS: Total and free PS ag (EIA, ELISA, Latex)
Coagulometric activity

CHARACTERIZATION : EIA, CIE
IMMUNOBLOTTING
Elisa

DNA SEQUENCING FOR GENE MUTATIONS

Similar hypercoagulable state and thrombosis risk in type I and type III protein S-deficient individuals from mixed type I/III families

Elisabetta Castoldi, Lisbeth F.A. Maurissen, Daniela Tormene, Luca Spiezia, Sabrina Gavasso, Claudia Radu, Tilman M. Hackeng, Jan Rosing, Paolo Simioni



Haematologica 2010

Factors commonly affecting measurment of protein C, protein S and antithrombin

Protein C activity **Chromogenic assay**	**Protein S** **Free protein S antigen**	**Antithrombin activity** **Chromogenic assay**
Physiological reduction	Physiological reduction	Physiological reduction
Neonates and children (different normal range from adults)	Neonates (Different normal range from adults)	Neonates (Different normal range from adults)
Other causes of reduction	Pregnancy and puerperium	Late pregnancy, early postpartum[a]
Vitamin K antagonists (e.g., warfarin)	Other causes of reduction	Other causes of reduction
Vitamin K deficiency	Vitamin K antagonists (e.g., warfarin)	Liver disease
Liver disease	Vitamin K deficiency	Disseminated intravascular coagulation
Disseminated intravascular coagulation	Liver disease	Nephrotic syndrome
Severe sepsis	Nephrotic syndrome	Severe sepsis
Artefactual increase	Disseminated intravascular coagulation	Recent thrombosis
DOACs or heparin if using clotting-based assay	Severe sepsis	Heparin therapy
Artefactual decrease	Recent thrombosis	L-asparaginase therapy
Factor V Leiden if using clotting-based assay	Oral oestrogen therapy (e.g., combined oral contraceptive pill or hormone therapy)	Artefactual increase
	Acute phase response	DOACs:
	Sickle cell disease	Xa inhibitors – if using Xa-based assay
	Artefactual increase	Thrombin inhibitors – if using thrombin-based assay
	DOACs or heparin if using clotting-based assay.	
	Artefactual decrease	
	Factor V Leiden if using clotting-based assay	

Arachchillage DJ, et al. Br J Haematol. 2022

Activated protein C resistance

Mutation in blood coagulation factor V associated with resistance to activated protein C

Rogier M. Bertina*, Bobby P. C. Koeleman*, Ted Koster†, Frits R. Rosendaal*†, Richard J. Dirven*, Hans de Ronde*, Pieter A. van der Velden* & Pieter H. Reitsma*

* Hemostasis and Thrombosis Research Center, and
† Department of Clinical Epidemiology, University Hospital, Bldg 1-C2R, PO Box 9600, 2300 RC Leiden, The Netherlands

Nature, 1994

Factor V Leiden mutation

- First described in 1994
- Missense mutation on fV gene on chromosome 1 causing switch of glutamine with arginine (Arg506Gln), thus making fV more resistent to inactivation from C activated protein
- Accounting for almost 50% of inherited thrombophilias
- 3-6% of Europeans and 6% of American white people.
- Several point mutations in the *F5 gene causing APC resistance have been identified in* different populations, including Arg306→Thr (FV Cambridge), Arg306→Gly (FV Hong Kong), Ile359→Thr (FV Liverpool), Glu666→Asp (mechanism unknown), and Ala512→Val (FV Bonn)

Distribution of fV Leiden in Caucasian population

	Heterozygosis	Homozygosis
General population	2-5%	0,1%
Patients with VTE	10-20%	1%
Families with thrombophilia	40-50%	6%

Modified from Rodeghiero et al, Ann Intern Med 1999

Risk of fV Leiden- associated thrombosis

- 3-8 fold greater in comparison with general population for heterozygus subjects, up to 80 fold increase for homozygous subjects with regards to venous thromboembolism
- **Interestingly, heterozygosis has a moderate impact on the risk of recurrence after a first episode VTE**
- **Homozygosis has a presumed increased risk compared to heterozygosis, even if data are discordant**
- There is not a definite increase in risk for arterial thrombosis

Campello et al, Expert Rev Hematology, 2016

Kujovich, Factor V Leiden trombophilia, Genet Med 2011

Table 3 Estimated risk of thrombotic complications: Factor V Leiden heterozygotes

Thrombotic complication	Estimated risk (odds ratio)[a]
First VTE[61–64]	3–8
Cerebral vein thrombosis[46,59]	3–5
Primary upper extremity thrombosis[b][43,45]	3–6
CVC-associated thrombosis[65]	2–3
Superficial vein thrombosis[60]	6
Pregnancy-associated VTE[66,67]	8–52
Recurrent VTE[42,68]	1.4–1.6
Pregnancy loss[66,69–71]	2–4

[a]Risk relative to individuals without Factor V Leiden.
[b]Not related to malignancy or a central venous catheter.
VTE, venous thromboembolism; CVC, central venous catheter.

Table 4 Estimated risk of thrombotic complications: Factor V Leiden homozygotes

Thrombotic complication	Risk (odds ratio)[a]
First VTE[4,33,62]	10–80
Pregnancy-associated VTE[66,73,74]	20–40
Oral contraceptive-associated VTE[75]	100
Recurrent VTE[33]	2–3
Surgery-associated VTE[76]	20
Early fetal loss[66]	3
Late fetal loss[b][77]	11

[a]Risk relative to individuals without a Factor V Leiden allele.
[b]Occurring after 12 weeks gestation.
VTE, venous thromboembolism.

From Kujovich, Genet Med 2011

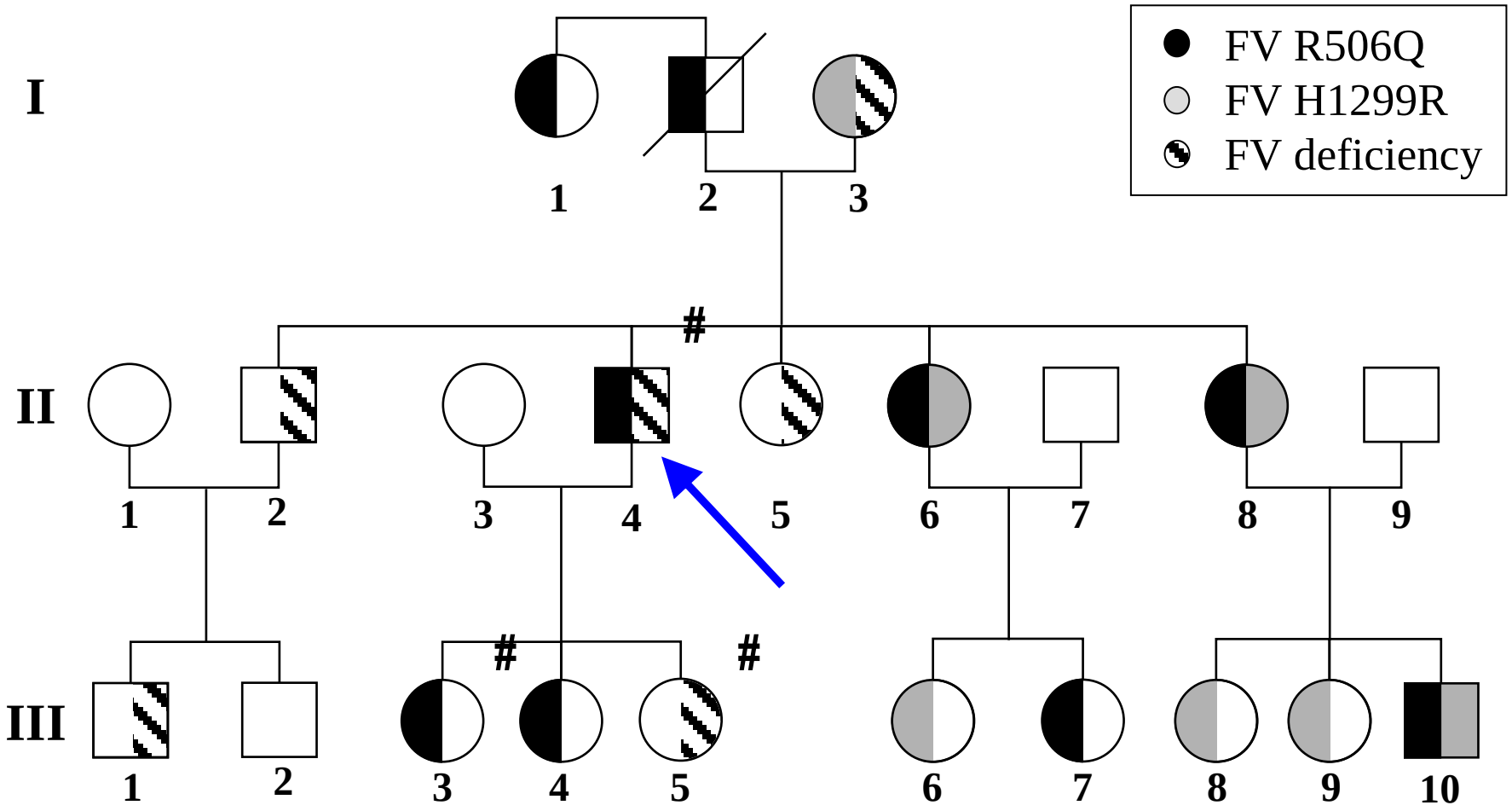
Table 5 Estimated risk of venous thromboembolism in Factor V Leiden heterozygotes with coexisting risk factors

Coexisting risk factor	Risk of VTE (odds ratio)[a]
PG20210G>A double heterozygote[48,108]	20 100 (pregnancy-associated VTE)
Hyperhomocysteinemia[109]	22
Obesity[110]	8
Oral contraceptives[46,111–114]	11–41 30 (cerebral vein thrombosis)
Third generation oral contraceptives[b114]	50
HRT[115–118]	7–16
Air travel[112,119]	14–16
Minor injury[57]	50
Malignancy[45,120]	12 20 (upper extremity thrombosis)

[a]Risk relative to risk of individuals without either risk factor.
[b]Oral contraceptives containing the third-generation progestagen desogestrel.
VTE, venous thromboembolism; PG 20210G>A, prothrombin 20210 G>A mutation; HRT, hormone replacement therapy.

FV Leiden pseudohomozygosis

- Heterozygous FVL carriers with concomitant heterozygous F5 mutation causing fV deficiency, resulting in 50% of FV plasma levels being all FVL.
- This condition leads to severe resistance to APC and thus to a thrombotic risk comparable to fVL homozygous



 # prothrombin G20210A

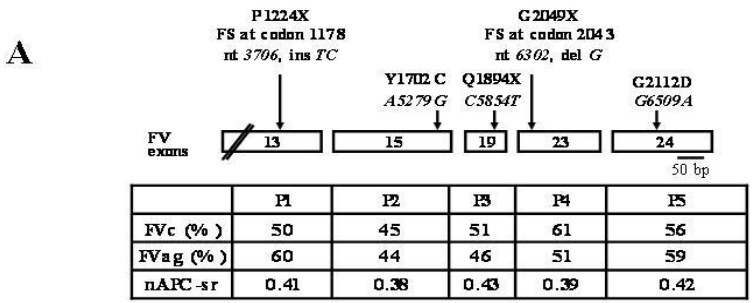
Castoldi et al, Blood 2000

Brief report

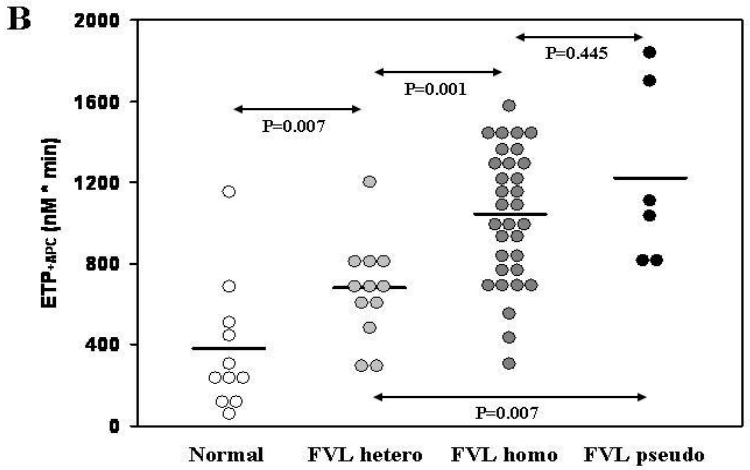
An underestimated combination of opposites resulting in enhanced thrombotic tendency

Paolo Simioni, Elisabetta Castoldi, Barbara Lunghi, Daniela Tormene, Jan Rosing, and Francesco Bernardi

Figure 1

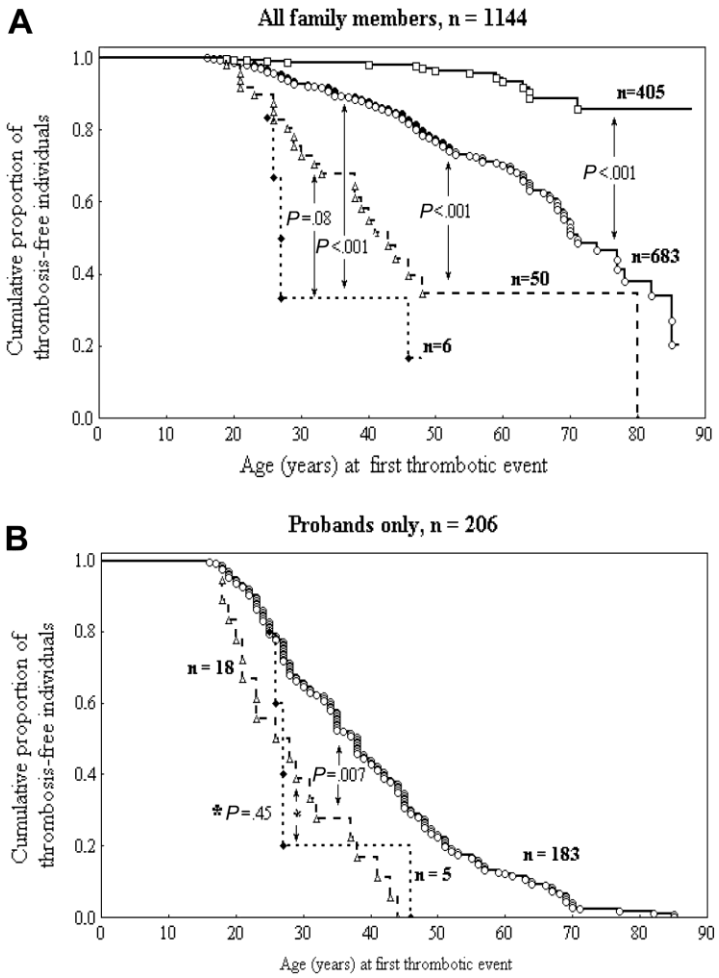


	P1	P2	P3	P4	P5
FVc (%)	50	45	51	61	56
FVag (%)	60	44	46	51	59
nAPC-sr	0.41	0.38	0.43	0.39	0.42



Blood 2005

Risk of VTE in FVL pseudohomozygosis and survival-free rates



Thrombosis-free survival curves of individuals with different FV genotypes. (A) Kaplan-Meier thrombosis-free survival analysis of the whole study cohort (probands and family members). (B) Kaplan-Meier thrombosis-free survival analysis of probands only. □, noncarriers; ○, FV Leiden heterozygotes; ▵, FV Leiden homozygotes; ♦, FV Leiden pseudohomozygotes. Differences between the curves were evaluated with the log-rank test. The apparently solid circles in the curve for FV Leiden heterozygotes are the effect of partial overlapping of open circles.

Paolo Simioni,Elisabetta Castoldi,Barbara Lunghi,Daniela Tormene,Jan Rosing,Francesco Bernardi, An underestimated combination of opposites resulting in enhanced thrombotic tendency, Blood, 2005, Figure 2.

Prothrombin variant

- G to A nucleotide change at 20210 in the 3'-untranslated region of the prothrombin (*F2) gene (PT G20210A).* PT G20210A predisposes to VTE either by promoting thrombin generation or by inhibiting factor Va inactivation by APC, thus creating indirectly APC resistance
- 0.7-6,5% in heterozygosis
- Most in Southern European population
- Almost absent in black or Asian people
- Probable founder effect as described for fVLeiden mutation

Prothrombin variant-associated thrombosis

- Heterozygosis has an increased risk (2-3 fold), but we do not have sufficient data for homozygosis, probably 5-fold (even if we can suppose it is increased)
- With regards to recurrent VTE, we do not have definite data

Mc Callum, BMJ 2014

Shemesh, Am J Hematol 2017

And what about carriers of both mutations?

Data are discordant: according to Martinelli et al there is a six-time

higher risk than relatives without mutations, while according to

Emmerich there is only a slight increase probably due to different

selection of populations and study design

Risk of recurrence for DVT in patients with double mutation

TABLE 2. RELATIVE RISK OF RECURRENT DEEP VENOUS THROMBOSIS.*

VARIABLE	PATIENTS WHO WERE HETEROZYGOUS FOR FACTOR V LEIDEN AND G20210A PROTHROMBIN MUTATION (N=17)					PATIENTS WHO WERE HETEROZYGOUS FOR FACTOR V LEIDEN (N=112)			PATIENTS WITH NEITHER MUTATION (N=283)
	INCIDENCE	RELATIVE RISK (95% CI)†	P VALUE	RELATIVE RISK (95% CI)‡	P VALUE	INCIDENCE	RELATIVE RISK (95% CI)‡	P VALUE	INCIDENCE
	no. (%)					no. (%)			no. (%)
Recurrent DVT	11 (65)	2.6 (1.3–5.1)	0.002	2.7 (1.4–5.0)	<0.001	34 (30)	1.1 (0.7–1.6)	0.76	86 (30)
Spontaneous recurrent DVT	10 (59)	3.7 (1.7–7.7)	<0.001	3.4 (1.7–6.6)	<0.001	23 (21)	1.0 (0.6–1.6)	0.81	59 (21)
Spontaneous recurrent DVT after a spontaneous first episode of DVT§	7 (88)	5.4 (2.0–14.1)	<0.001	5.1 (2.2–11.4)	<0.001	10 (24)	1.0 (0.5–2.0)	0.97	33 (29)
Spontaneous recurrent DVT after a secondary first episode of DVT¶	3 (33)	2.1 (0.6–7.3)	0.22	1.9 (0.6–6.2)	0.26	13 (18)	1.2 (0.6–2.3)	0.65	26 (15)

*The P values were calculated by the log-rank test. CI denotes confidence interval, and DVT deep venous thrombosis.

†The comparison group is the group of patients who were heterozygous for factor V Leiden.

‡The comparison group is the group of patients with neither mutation.

§The percentages are calculated on the basis of the total number of spontaneous first episodes of deep venous thrombosis: 8 among the patients who were heterozygous for both mutations, 41 among those who were heterozygous for factor V Leiden, and 114 among those with neither mutation.

¶The percentages are calculated on the basis of the total number of secondary first episodes of deep venous thrombosis: 9 among the patients who were heterozygous for both mutations, 71 among those who were heterozygous for factor V Leiden, and 169 among those with neither mutation.

From De Stefano et al, NEJM, 1999

ORIGINAL ARTICLE

The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A

I. MARTINELLI,* T. BATTAGLIOLI,* V. DE STEFANO,† D. TORMENE,‡ L. VALDRÈ,§ E. GRANDONE,¶ A. TOSETTO** and P. M. MANNUCCI*, ON BEHALF OF THE GIT (GRUPPO ITALIANO TROMBOFILIA)

*A. Bianchi Bonomi Haemophilia and Thrombosis Center, Department of Internal Medicine and Medical Specialties, University of Milan and IRCCS Ospedale Maggiore Policlinico, Mangiagalli and Regina Elena Foundation, Milan; †Institute of Hematology, Catholic University, Rome; ‡Department of Medical and Surgical Sciences, University of Padua, Padua; §Department of Angiology and Blood Coagulation M. Golinelli, S. Orsola-Malpighi University Hospital, Bologna; ¶Atherosclerosis and Thrombosis Unit, IRCCS Casa Sollievo della Soerenza, San Giovanni Rotondo; and **Hematology Department, S. Bortolo Hospital, Vicenza, Italy

Table 2 Number and rate of VTEs and age at occurrence in combined heterozygous factor V Leiden and prothrombin G20210A, single heterozygous factor V Leiden or prothrombin G20210A and women without thrombophilia

	Factor V Leiden and prothrombin G20210A	Factor V Leiden	Prothrombin G20210A	No thrombophilia
VTE, *n* – % (95% CI)				
Pregnancy	0–0 (0–2.7)	0–0 (0–1.5)	0–0 (0–1.5)	0–0 (0–1.4)
Puerperium	2–1.8 (0.5–6.3)	3–1.5 (0.5–4.3)	2–1 (0.2–3.6)	1–0.4 (0–2.5)
Median age (range) at VTE, years	33 (30–35)	34 (27–35)	27 (25–31)	38

Journal of Thrombosis and Haemostasis, 2008

Factor VIII

fVIII (U/dl)	Patients	Controls	OR (CI 95%)
<100	52	111	1
100-124	88	96	2,3 (1,3-3,8)
125-149	85	60	3 (1,6-5,7)
>150	76	34	4,8 (2,3-10)

Odds ratio adjusted for blood group and vWF levels.

Modified from Koster et al, 1995

fVIII, thrombosis and hormonal therapy

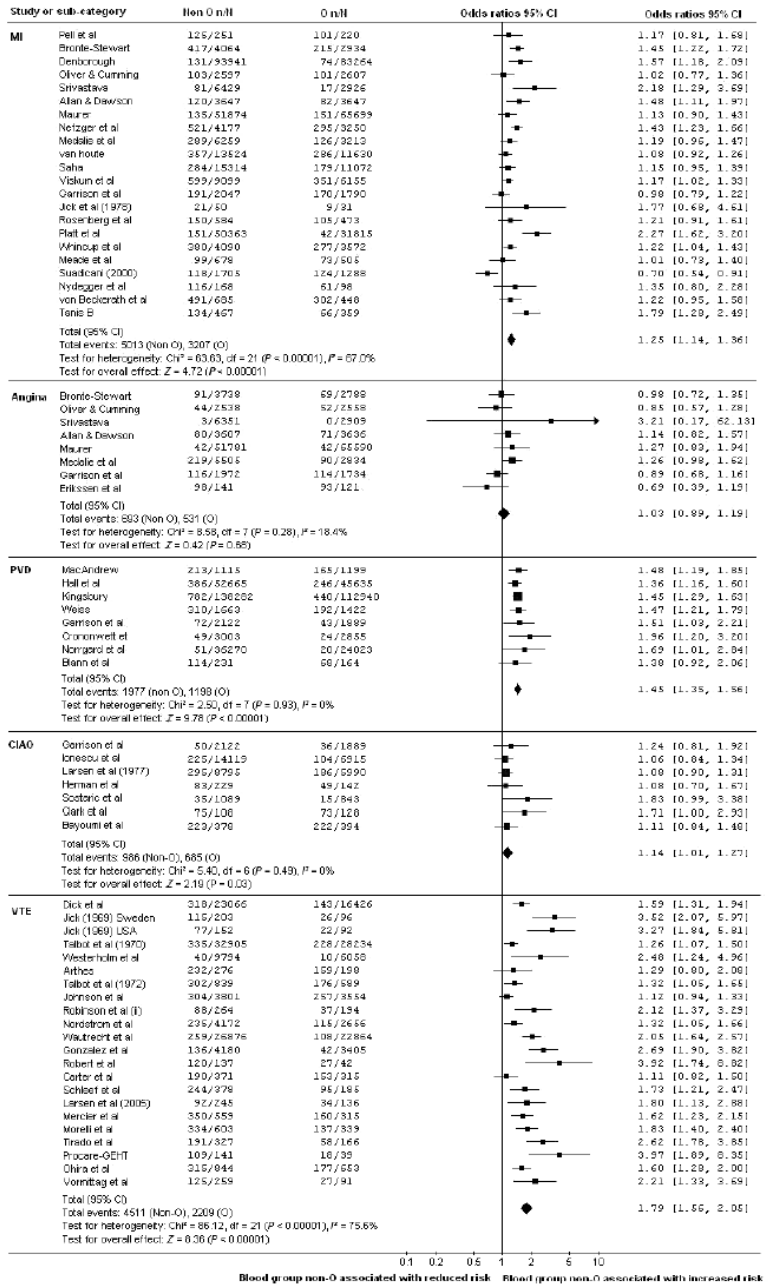
	Patients	Controls	Odds Ratio (CI95%)
OC - fVIII -	7	28	1
OC + fVIII -	13	13	4 (1.3-12.4)
OC - fVIII +	20	15	5.3 (1.8-15.5)
OC + fVIII +	36	14	10.3 (3.7-28.9)

OC= Oral contraceptives

Adapted from Bloemenkamp et al, 1999

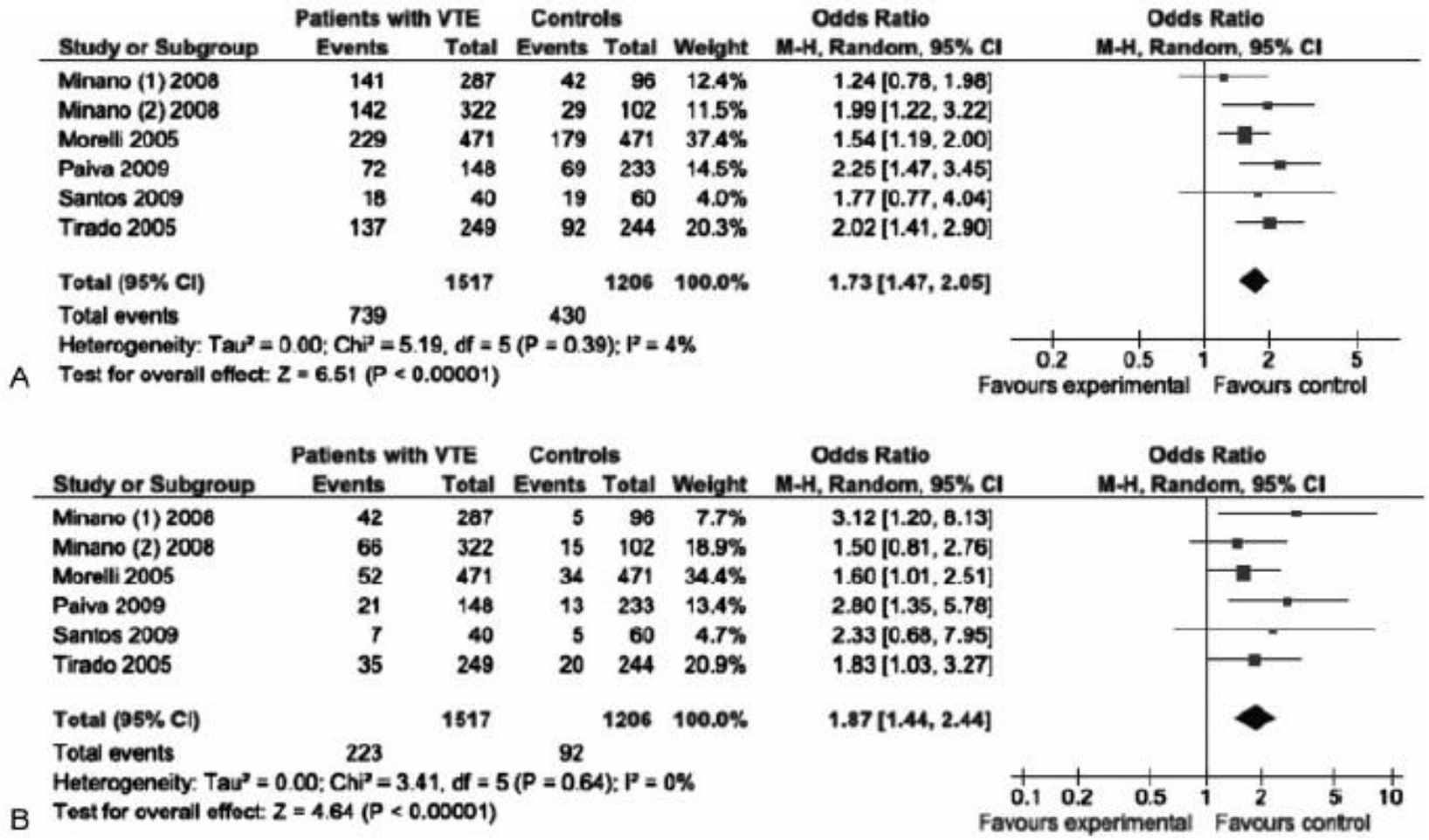
Blood group and the risk of VTE

Wu and colleagues performed a meta-analysis which selected 4709 VTE cases from 21 studies, and found an OR of 1.79 (95% confidence interval [CI] (1.56-0.05) in non-O versus O status



Wu et al, JTH 2008

Blood group and the risk of VTE



Study or Subgroup	Patients with VTE Events	Total	Controls Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Minano (1) 2008	141	287	42	96	12.4%	1.24 [0.78, 1.98]
Minano (2) 2008	142	322	29	102	11.5%	1.99 [1.22, 3.22]
Morelli 2005	229	471	179	471	37.4%	1.54 [1.19, 2.00]
Paiva 2009	72	148	69	233	14.5%	2.25 [1.47, 3.45]
Santos 2009	18	40	19	60	4.0%	1.77 [0.77, 4.04]
Tirado 2005	137	249	92	244	20.3%	2.02 [1.41, 2.90]
Total (95% CI)		1517		1206	100.0%	1.73 [1.47, 2.05]
Total events	739		430			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 5.19$, $df = 5$ ($P = 0.39$); $I^2 = 4\%$

Test for overall effect: $Z = 6.51$ ($P < 0.00001$)

Odds Ratio M-H, Random, 95% CI

0.2 0.5 1 2 5

Favours experimental Favours control

A

Study or Subgroup	Patients with VTE Events	Total	Controls Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Minano (1) 2008	42	287	5	96	7.7%	3.12 [1.20, 8.13]
Minano (2) 2008	66	322	15	102	18.9%	1.50 [0.81, 2.76]
Morelli 2005	52	471	34	471	34.4%	1.60 [1.01, 2.51]
Paiva 2009	21	148	13	233	13.4%	2.80 [1.35, 5.78]
Santos 2009	7	40	5	60	4.7%	2.33 [0.68, 7.95]
Tirado 2005	35	249	20	244	20.9%	1.83 [1.03, 3.27]
Total (95% CI)		1517		1206	100.0%	1.87 [1.44, 2.44]
Total events	223		92			

Heterogeneity: $Tau^2 = 0.00$; $Chi^2 = 3.41$, $df = 5$ ($P = 0.64$); $I^2 = 0\%$

Test for overall effect: $Z = 4.64$ ($P < 0.00001$)

Odds Ratio M-H, Random, 95% CI

0.1 0.2 0.5 1 2 5 10

Favours experimental Favours control

B

A further meta-analysis considering 38 studies, hence 10,305 VTE cases, reported that non-O blood groups increase approximately twofold the risk of VTE (OR 2.08; 95%CI 1.83–2.37)

Dentali et al, Semin Thromb Haemost 2012

ABO blood groups and the risk of venous thrombosis in patients with inherited thrombophilia

Luca Spiezia[1], Elena Campello[1], Maria Bon[1], Tiziana Tison[2], Marta Milan[1], Paolo Simioni[1], Paolo Prandoni[1]

[1]Department of Cardiothoracic and Vascular Sciences; [2]Blood Transfusion Unit, University Hospital of Padua, Padua, Italy

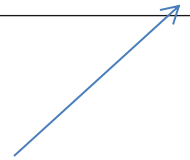
In conclusion, our data show that having a non-O blood group is associated with an increased risk of VTE and that the addition of thrombophilia increases the thrombotic risk conferred by non-O group alone by almost 3-fold. This simple information may help to identify groups of patients at high risk suitable for counselling, further testing or closer monitoring. Finally, this robust and easily assessable risk factor has the potential to be included -alone or in association with the determination of thrombophilia- as part of a more comprehensive risk assessment model for VTE.

Risk of DVT combining blood groups and inherited thrombophilia

Table II - Prevalence of blood groups in the study cases and controls (OR and 95% CI).

	Cases (n=712)	Controls (n=712)	OR (95% CI)
O	220 (30.9)	354 (49.7)	1*
Non-O	492 (69.1)	358 (50.3)	2.21 (1.78-2.75)
A	335 (47.0)	219 (30.7)	2.46 (1.94-3.13)
B	115 (16.2)	98 (13.8)	1.89 (1.37-2.59)
AB	42 (5.9)	41 (5.8)	1.65 (1.05-2.62)
O without thrombophilia	135 (18.9)	320 (44.9)	1*
O and thrombophilia	85 (11.9)	34 (4.8)	5.93 (3.79-9.25)
Non-O without thrombophilia	340 (47.8)	307 (43.1)	2.63 (2.04-3.38)
Non-O and thrombophilia	152 (21.4)	51 (7.2)	7.06 (4.85-10.28)

Legend *Reference category. Numbers in parentheses indicate percentages.



ibidem

Who?

Table 1. Clinical Characteristics Suggestive of Inherited Thrombophilia in Patients with Venous Thromboembolism (VTE).

Thrombosis at a young age (<50 yr), especially in association with weak provoking factors (minor surgery, combination oral contraceptives, or immobility) or unprovoked VTE
Strong family history of VTE (first-degree family members affected at a young age)
Recurrent VTE events, especially at a young age*
VTE in unusual sites such as splanchnic or cerebral veins†

* The antiphospholipid syndrome must also be considered, but it is not inherited.
† Patients with splanchnic-vein VTE should be assessed for myeloproliferative neoplasms and paroxysmal nocturnal hemoglobinuria.

Connors JM. NEJM 2017

Thrombophilia testing

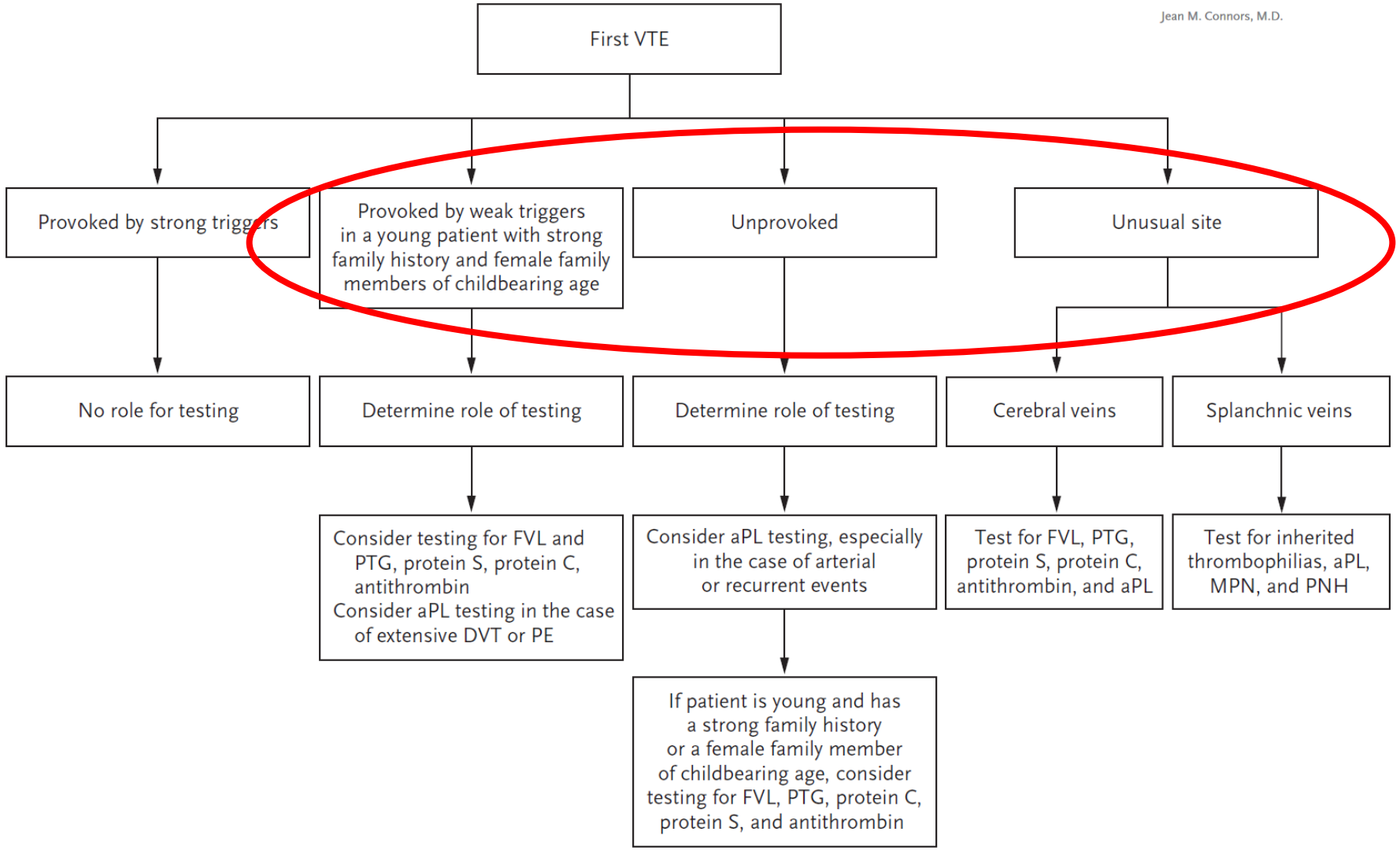
The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

Thrombophilia Testing and Venous Thrombosis

Jean M. Connors, M.D.



Connors JM. NEJM 2017 - adapted

WHY?
Reasons for screening VTE patients

1) To determine pathomechanism

2) Acute treatement not influenced by thrombophilia

- Rare peculiar cases of thrombophilic patients
  - tailor heparin in antithrombin deficiency
  - addition of antithrombin or protein C concentrates
  - antiphospholipid antibody syndrome and DOAC

3) Management of secondary prevention

4) Management of asymptomatic first-degree relatives

Connors JM. NEJM 2017

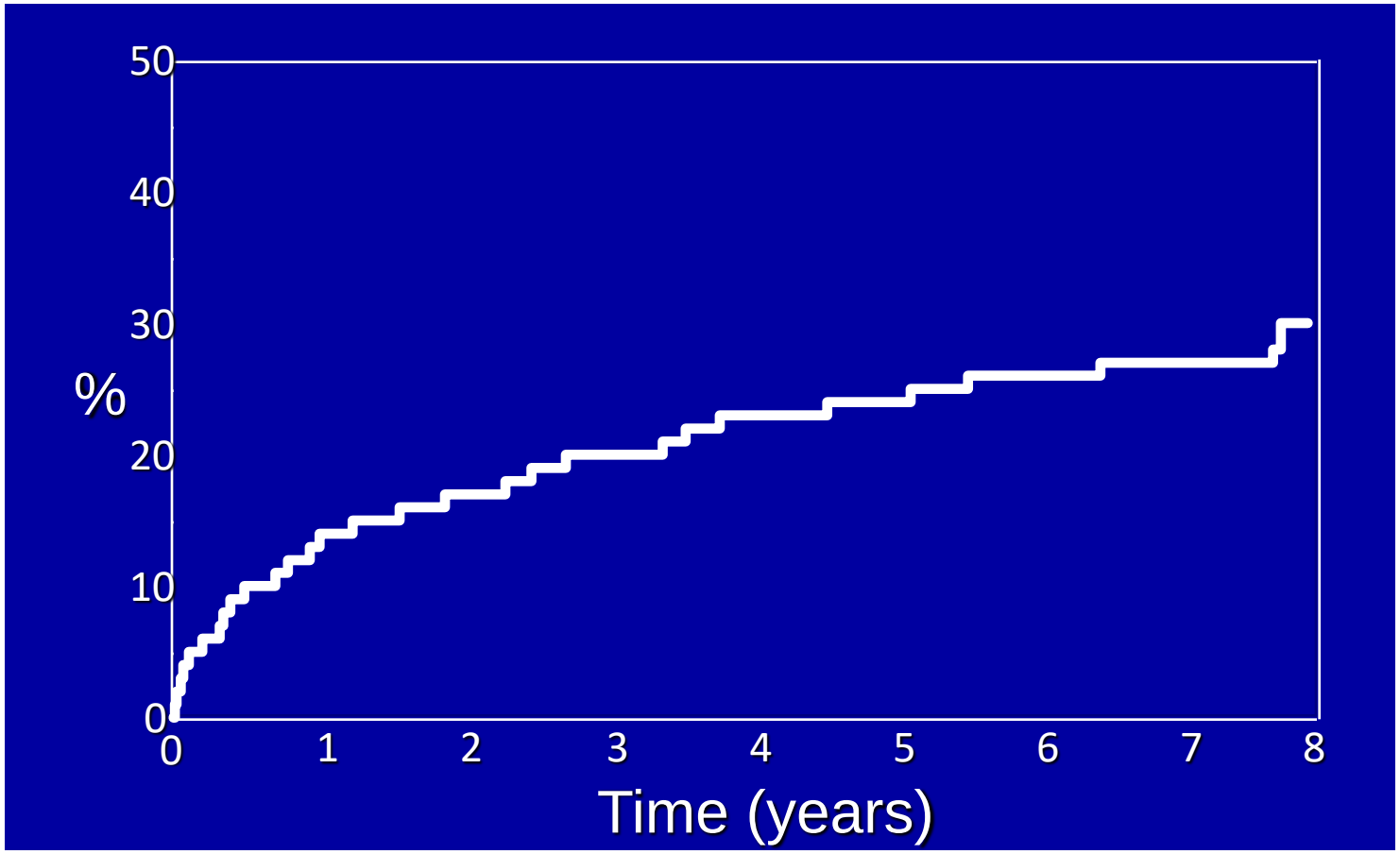
Optimal Duration of Anticoagulant Treatment in thrombophilic patients

- Clinical decision in an **individual patient** depends upon the estimated risks of VTE recurrence and treatment induced bleeding.

- Although the quality of the evidence in this area is low and does not allow firm recommendations, patients with **AT deficiency, homozygosity for FVL, multiple defects, and perhaps PC or PS deficiency, altered D-dimer** could be more prone to recurrence and therefore potential candidates for long term oral anticoagulation after a first unprovoked VTE.

 British Journal of Haematology, **149**, 209–220

Cumulative Incidence of VTE Recurrences (78/355)



Prandoni et al, Ann Intern Med 1996

Risk of Recurrent Venous Thromboembolism in Patients With Common Thrombophilia

A Systematic Review

Wai Khoon Ho, MBBS, FRACP; Graeme J. Hankey, MD, FRACP, FRCP; Daniel J. Quinlan, MBBS; John W. Eikelboom, MBBS, MSc, FRACP

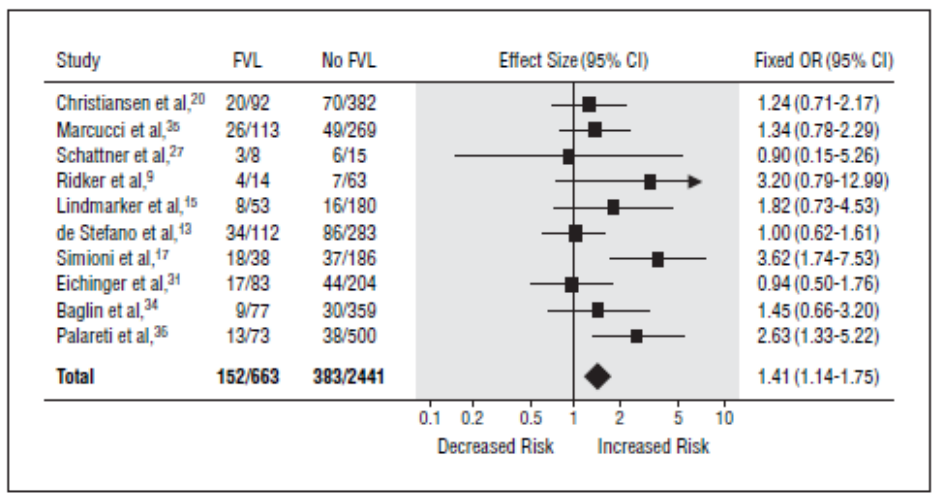


Figure 2. Risk of recurrent venous thromboembolism (VTE) and heterozygous factor V 1691A (Leiden) (FVL) vs no FVL in reviewed studies. Except where otherwise indicated, data are reported as number of patients with VTE recurrence/number of patients with a first VTE. In the test for heterogeneity, $\chi^2_9=15.35$, $P=.08$; $I^2=41.4\%$. For overall effect, $z=3.14$ ($P=.002$). In the graphic representation, squares represent the effect size; extended lines, 95% confidence interval (CI); and diamond, total effect size. The arrow on the standard error (SE) line of the Ridker et al[9] study indicates that the upper SE extends beyond the upper range of the illustrated scale by an unspecified amount. OR indicates odds ratio.

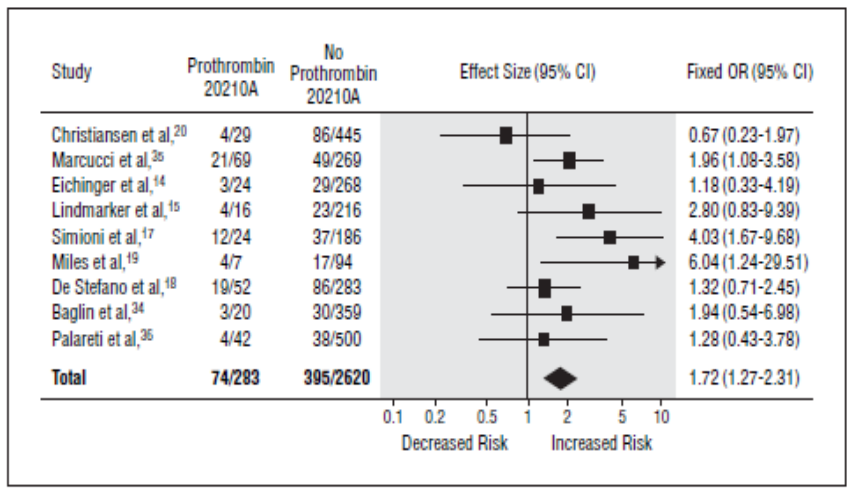


Figure 4. Risk of recurrent venous thromboembolism (VTE) and heterozygous prothrombin G20210A vs no G20210A in reviewed studies. Except where otherwise indicated, data are reported as number of patients with VTE recurrence/number of patients with a first VTE. In the test for heterogeneity, $\chi^2_8=11.14$, $P=.19$; $I^2=28.2\%$. For overall effect, $z=3.55$ ($P<.001$). In the graphic representation, squares represent the effect size; extended lines, confidence interval (CI); and diamond, total effect size. The arrow on the standard error (SE) line of the Miles et al[19] study indicates that the upper SE extends beyond the upper range of the illustrated scale by an unspecified amount. OR indicates odds ratio.

Predictive Value of D-Dimer Test for Recurrent Venous Thromboembolism After Anticoagulation Withdrawal in Subjects With a Previous Idiopathic Event and in Carriers of Congenital Thrombophilia

Gualtiero Palareti, MD; Cristina Legnani, MS; Benilde Cosmi, MD; Lelia Valdré, MD; Barbara Lunghi, MS; Francesco Bernardi, MS; Sergio Coccheri, MD

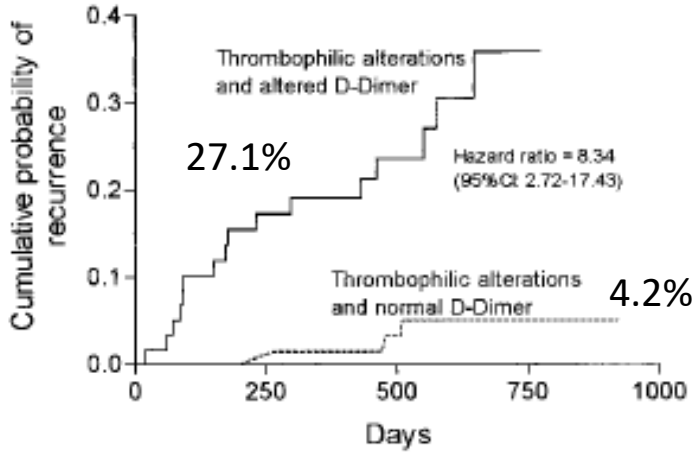


Figure 2. Cumulative probability of VTE recurrence in subjects with congenital thrombophilic alterations according to normal (≤500 ng/mL) or altered (>500 ng/mL) D-dimer results obtained 1 month after anticoagulation was stopped.

TABLE 3. Multivariate Regression Analysis of Relative Risks of Altered D-Dimer Results Obtained in All Subjects and in Subgroups

	Relative Risk (95% CI)*	*P*
All subjects	2.61 (1.45–4.71)	0.001
Idiopathic index event	2.75 (1.24–6.12)	0.013
Cancer-associated index event	2.96 (0.80–10.88)	0.103
Secondary index event	1.90 (0.50–7.28)	0.349
With thrombophilic alterations	5.88 (1.46–23.72)	0.013
Without thrombophilic alterations	2.19 (1.10–4.35)	0.026

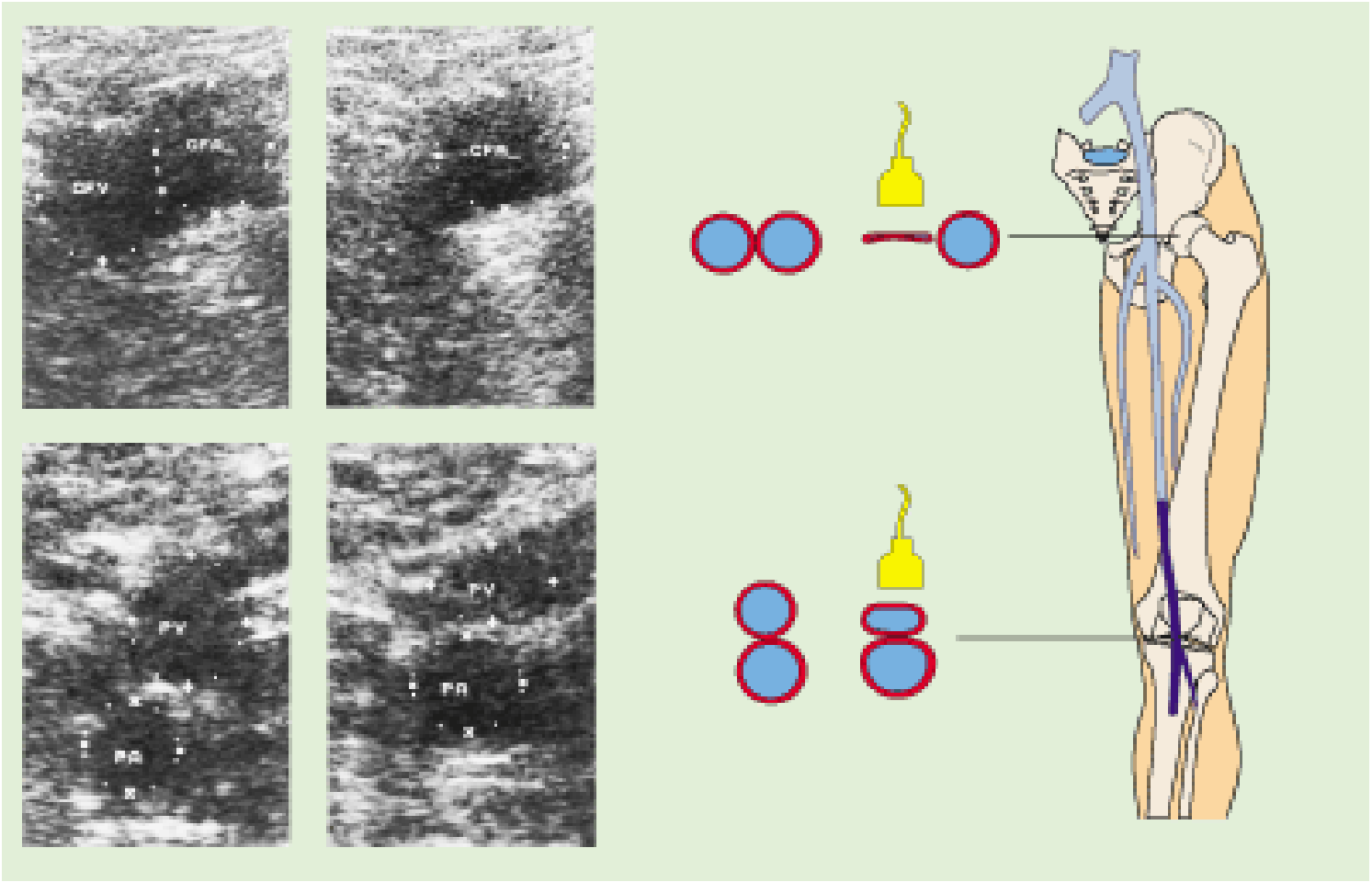
*Risks were calculated with adjustment for age, sex, duration of previous oral anticoagulant treatment, and presence/absence of congenital thrombophilic alterations or nature of index event, as appropriate.

Circulation. 2003;108:313-318

Deep-vein thrombosis

Lensing et al Lancet 1999:353:479

- Plaatje



Residual vein obstruction to predict the risk of recurrent venous thromboembolism in patients with deep vein thrombosis: a systematic review and meta-analysis

M. CARRIER,*† M. A. RODGER,*† P. S. WELLS,*† M. RIGHINI‡§ and G. LE GAL¶

*Thrombosis Program, Division of Hematology, Department of Medicine, University of Ottawa; †Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, ON, Canada; Divisions of ‡Angiology and Hemostasis and §General Internal Medicine, Geneva University Hospital and Faculty of Medicine, Geneva, Switzerland; and ¶Department of Internal Medicine and Chest Diseases, EA3878, Brest University Hospital, Brest, France

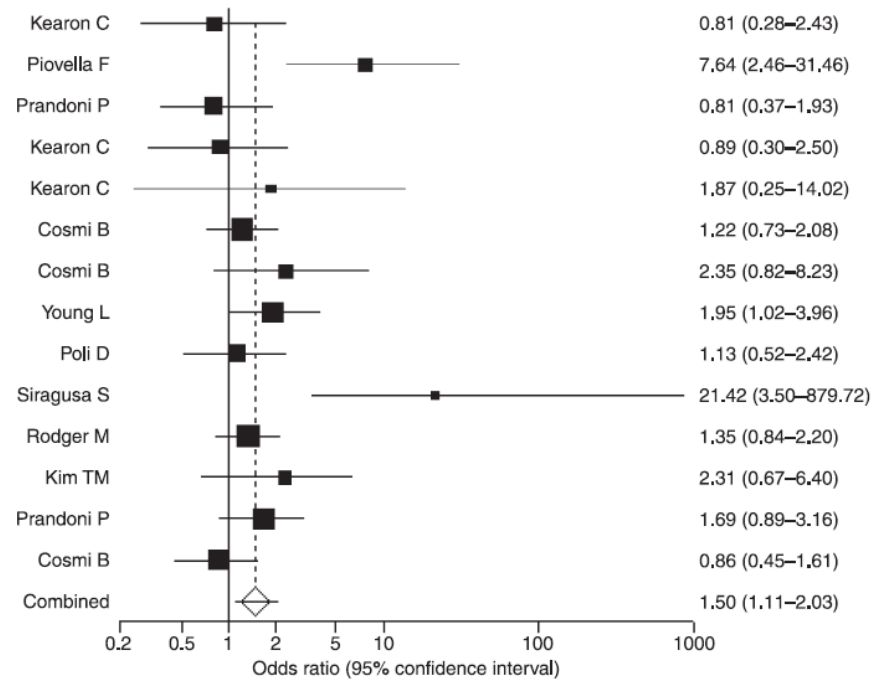


Fig. 1. Forest plot and pooled estimates of the odds ratios evaluating the association between residual vein obstruction and recurrent venous thromboembolism in patients with a first episode of deep vein thrombosis (provoked or unprovoked) following at least 3 months of anticoagulation.

Effect of patient's sex on risk of recurrent venous thromboembolism: a meta-analysis

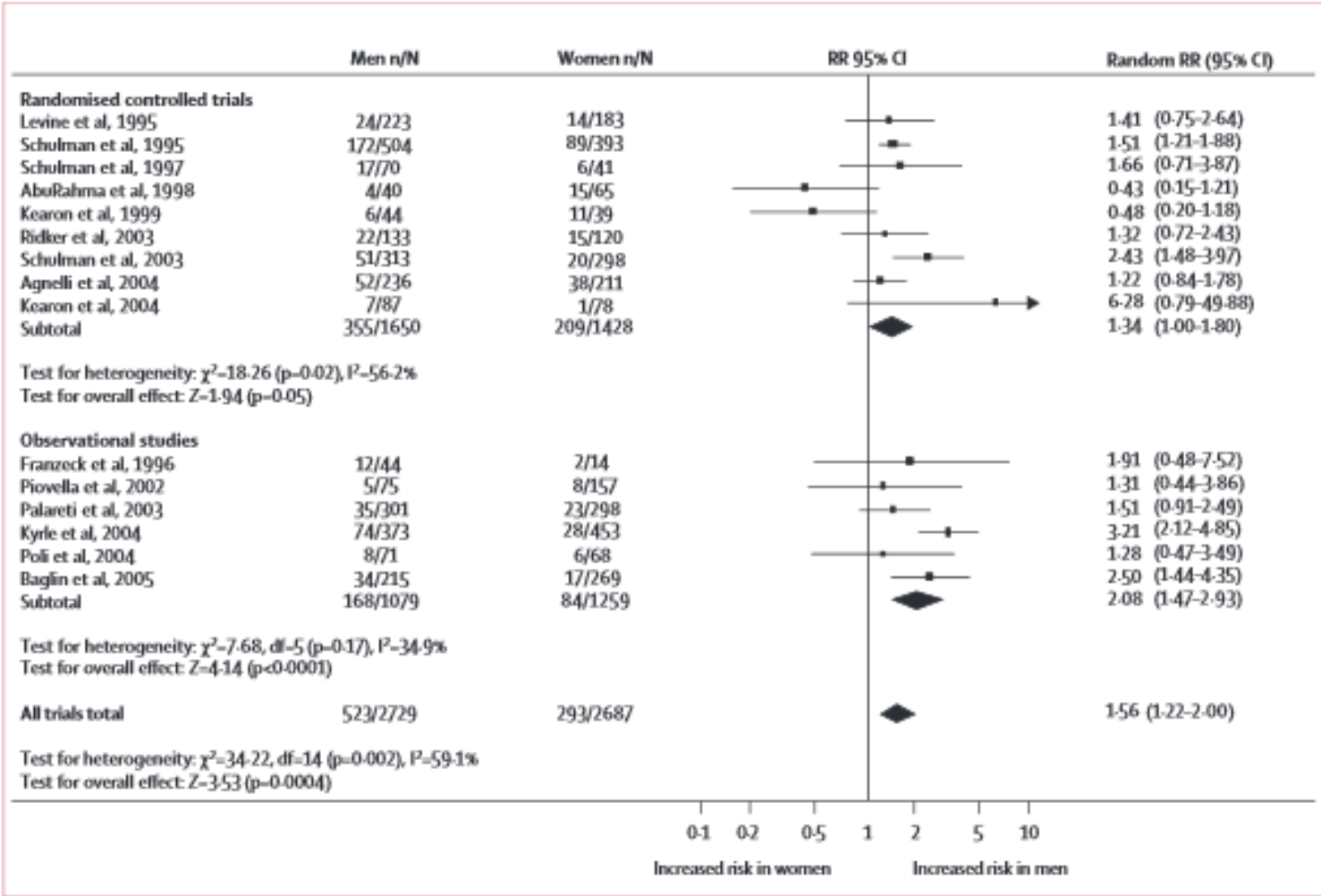


Figure 2: Risk of recurrent venous thromboembolism in men compared with in women

Overweight, Obesity, and the Risk of Recurrent Venous Thromboembolism

Sabine Eichinger, MD; Gregor Hron, MD; Christine Bialonczyk, MD; Mirko Hirschl, MD; Erich Minar, MD; Oswald Wagner, MD; Georg Heinze, PhD; Paul A. Kyrle, MD

Table 2. Relative Risk of Recurrent Venous Thromboembolism According to Categories of Body Weight

Body Weight Category	Patients/ Events, No.	Relative Risk (95% CI)	
		Unadjusted	Adjusted[a]
Normal weight	416/44	1 [Reference]	1 [Reference]
Overweight	420/74	1.7 (1.2-2.5)	1.3 (0.9-1.9)
Obese	271/50	1.9 (1.2-2.8)	1.6 (1.0-2.4)

Abbreviation: CI, confidence interval.
[a]Adjusted for age, sex, factor V Leiden, prothrombin G20210A mutation, high factor VIII level, and type of initial venous thromboembolic event.

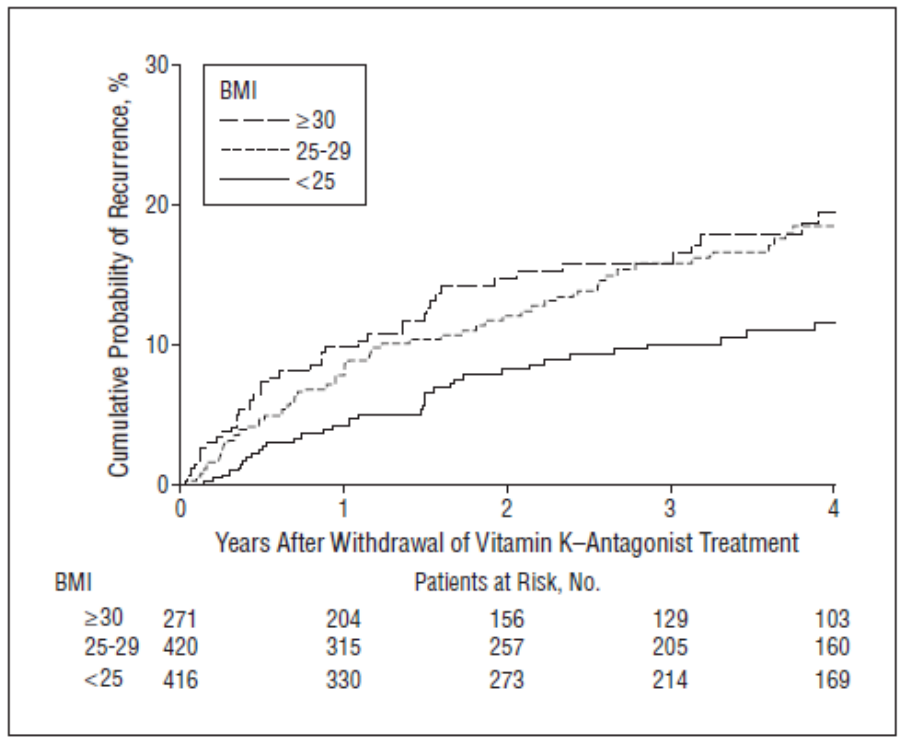
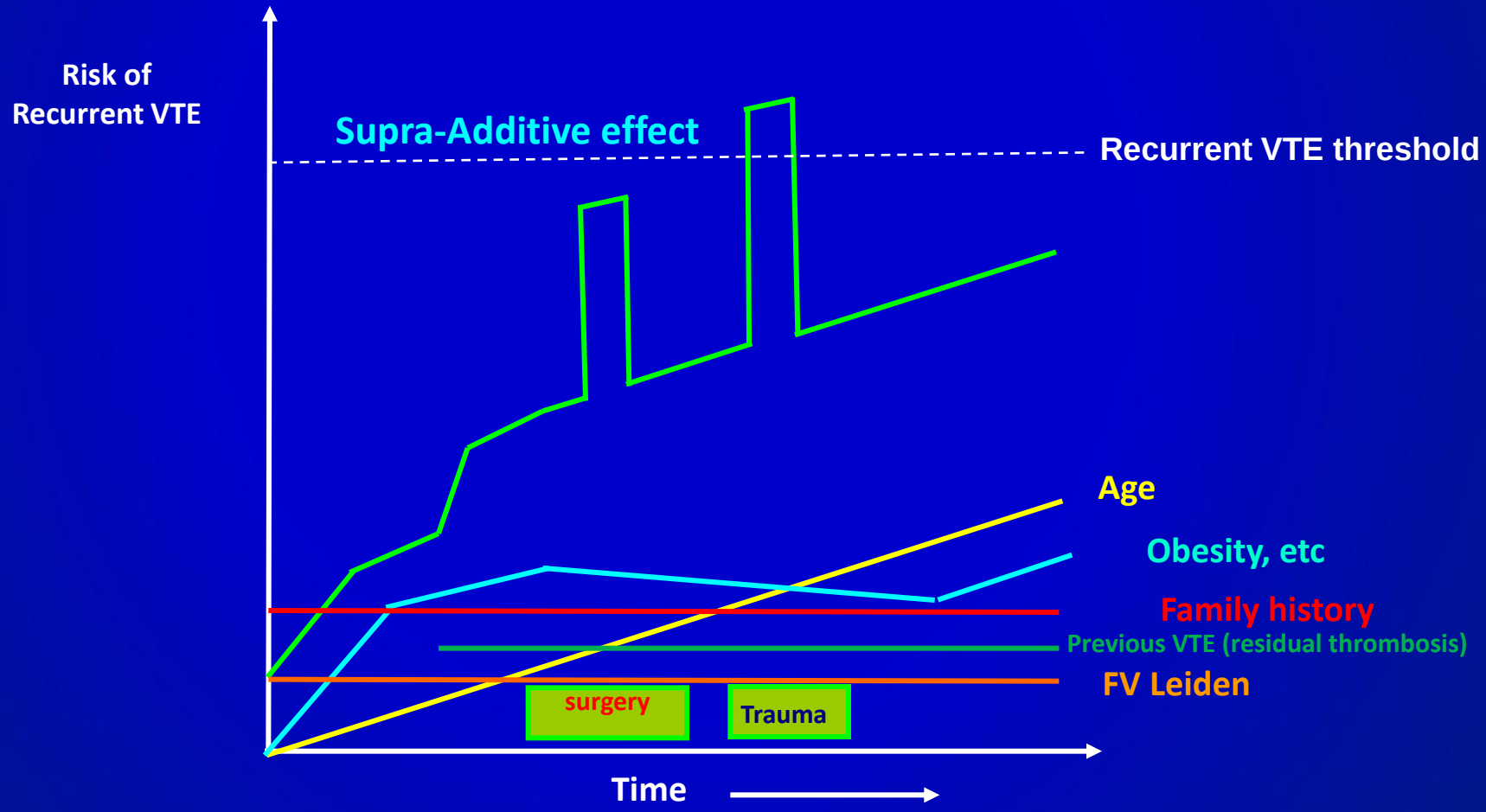


Figure 2. Kaplan-Meier estimates of the risk of recurrent venous thromboembolism in patients according to categories of body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared).

Arch Intern Med. 2008;168(15):1678-1683

A MULTIFACTORIAL MODEL FOR RECURRENT THROMBOSIS



Optimal Duration of Anticoagulant Treatment in thrombophilic patients

Table 11 Categorization of risk factors for venous thromboembolism based on the risk of recurrence over the long-term

Estimated risk for long-term recurrence[a]	Risk factor category for index PE[b]	Examples[b]
Low (<3% per year)	Major transient or reversible factors associated with >10-fold increased risk for the index VTE event (compared to patients without the risk factor)	• Surgery with general anaesthesia for >30 min • Confined to bed in hospital (only "bathroom privileges") for ≥3 days due to an acute illness, or acute exacerbation of a chronic illness • Trauma with fractures
Intermediate (3–8% per year)	Transient or reversible factors associated with ≤10-fold increased risk for first (index) VTE	• Minor surgery (general anaesthesia for <30 min) • Admission to hospital for <3 days with an acute illness • Oestrogen therapy/contraception • Pregnancy or puerperium • Confined to bed out of hospital for ≥3 days with an acute illness • Leg injury (without fracture) associated with reduced mobility for ≥3 days • Long-haul flight
	Non-malignant persistent risk factors	• Inflammatory bowel disease • Active autoimmune disease
	No identifiable risk factor	
High (>8% per year)		• Active cancer • One or more previous episodes of VTE in the absence of a major transient or reversible factor • Antiphospholipid antibody syndrome

Carriers of severe hereditary thrombophilia (antithrombin, *protein C or protein S deficiencies, homozygous factor V Leiden or prothrombin G20210A*)

Konstantinides SV et al ESC 2020

Who?

NICE National Institute for Health and Care Excellence

Venous thromboembolic diseases: diagnosis, management and thrombophilia testing

NICE guideline
Published: 26 March 2020
www.nice.org.uk/guidance/ng158

NICE guideline

1.9 Thrombophilia testing

1.9.1 Do not offer testing for hereditary thrombophilia to people who are continuing anticoagulation treatment. [2012, amended 2020]

1.9.2 Do not offer thrombophilia testing to people who have had provoked DVT or PE. [2012] !?

1.9.3 Consider testing for antiphospholipid antibodies in people who have had unprovoked DVT or PE if it is planned to stop anticoagulation treatment, but be aware that these tests can be affected by anticoagulants and specialist advice may be needed. [2012, amended 2020]

1.9.4 Consider testing for hereditary thrombophilia in people who have had unprovoked DVT or PE and who have a first-degree relative who has had DVT or PE if it is planned to stop anticoagulation treatment, but be aware that these tests can be affected by anticoagulants and specialist advice may be needed. [2012, amended 2020]

1.9.5 Do not routinely offer thrombophilia testing to first-degree relatives of people with a history of DVT or PE and thrombophilia. [2012] !?

American Society of Hematology 2023 guidelines for management of venous thromboembolism: thrombophilia testing

Clinical scenarios for thrombophilia testing

Saskia Middeldorp,[1] Robby Nieuwlaat,[2,3] Lisa Baumann Kreuziger,[4] Michiel Coppens,[5,6] Damon Houghton,[7,8] Andra H. James,[9] Eddy Lang,[10] Stephan Moll,[11] Tarra Myers,[12] Meha Bhatt,[2] Chatree Chai-Adisaksopha,[13] Luis E. Colunga-Lozano,[14] Samer G. Karam,[2,3] Yuan Zhang,[1,2] Wojtek Wiercioch,[2,3] Holger J. Schünemann,[2,3,15] and Alfonso Iorio[3]

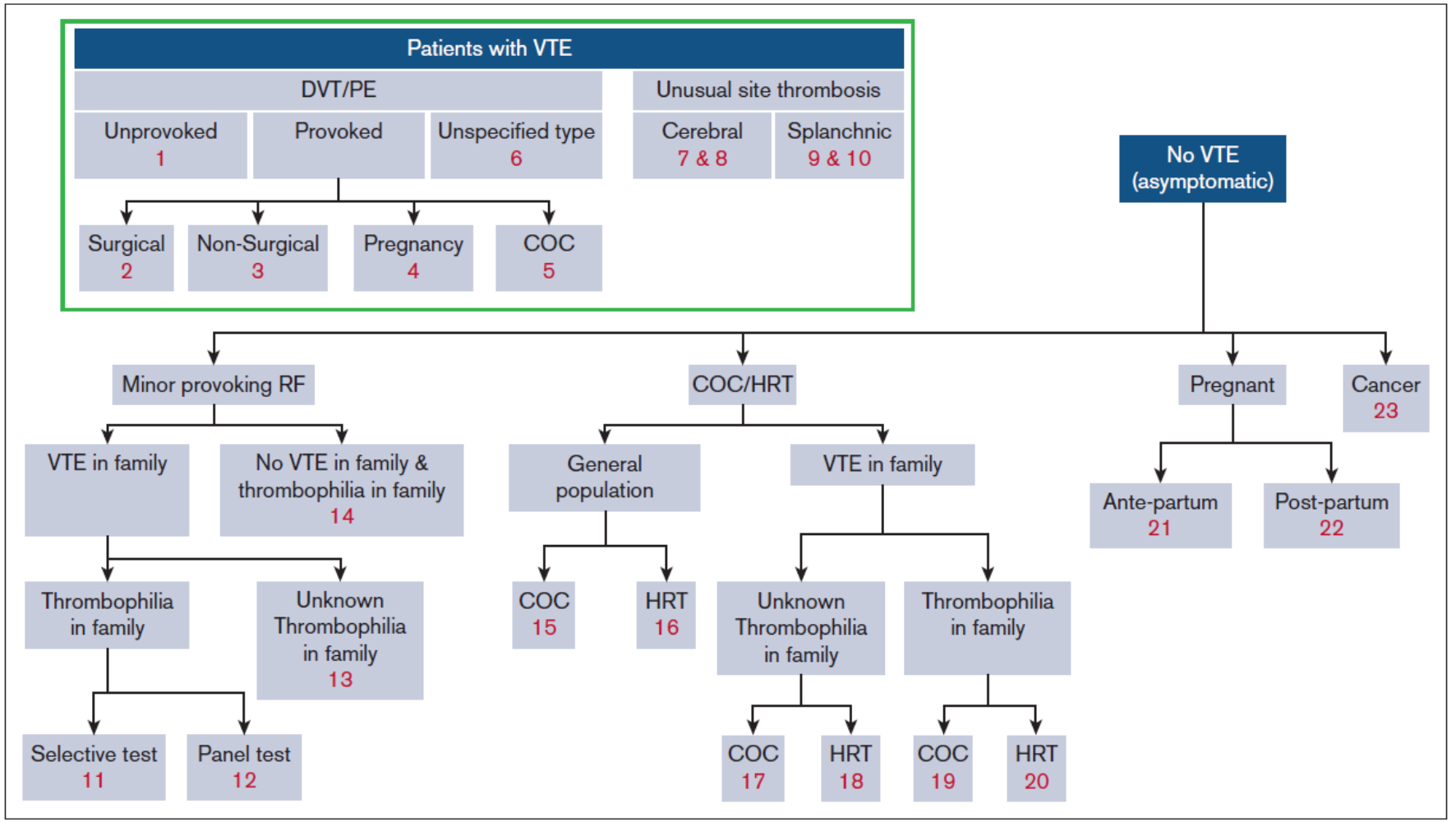


Figure 1. Overview of guideline questions. Minor provoking risk factors: circumstances that generally do not require prophylaxis, such as immobility or minor injury, illness, or infection. RF, risk factor.

American Society of Hematology 2023 guidelines for management of venous thromboembolism: thrombophilia testing

Saskia Middeldorp,[1] Robby Nieuwlaat,[2,3] Lisa Baumann Kreuziger,[4] Michiel Coppens,[5,6] Damon Houghton,[7,8] Andra H. James,[9] Eddy Lang,[10] Stephan Moll,[11] Tarra Myers,[12] Meha Bhatt,[2] Chatree Chai-Adisaksopha,[13] Luis E. Colunga-Lozano,[14] Samer G. Karam,[2,3] Yuan Zhang,[1,2] Wojtek Wiercioch,[2,3] Holger J. Schünemann,[2,3,15] and Alfonso Iorio[3]

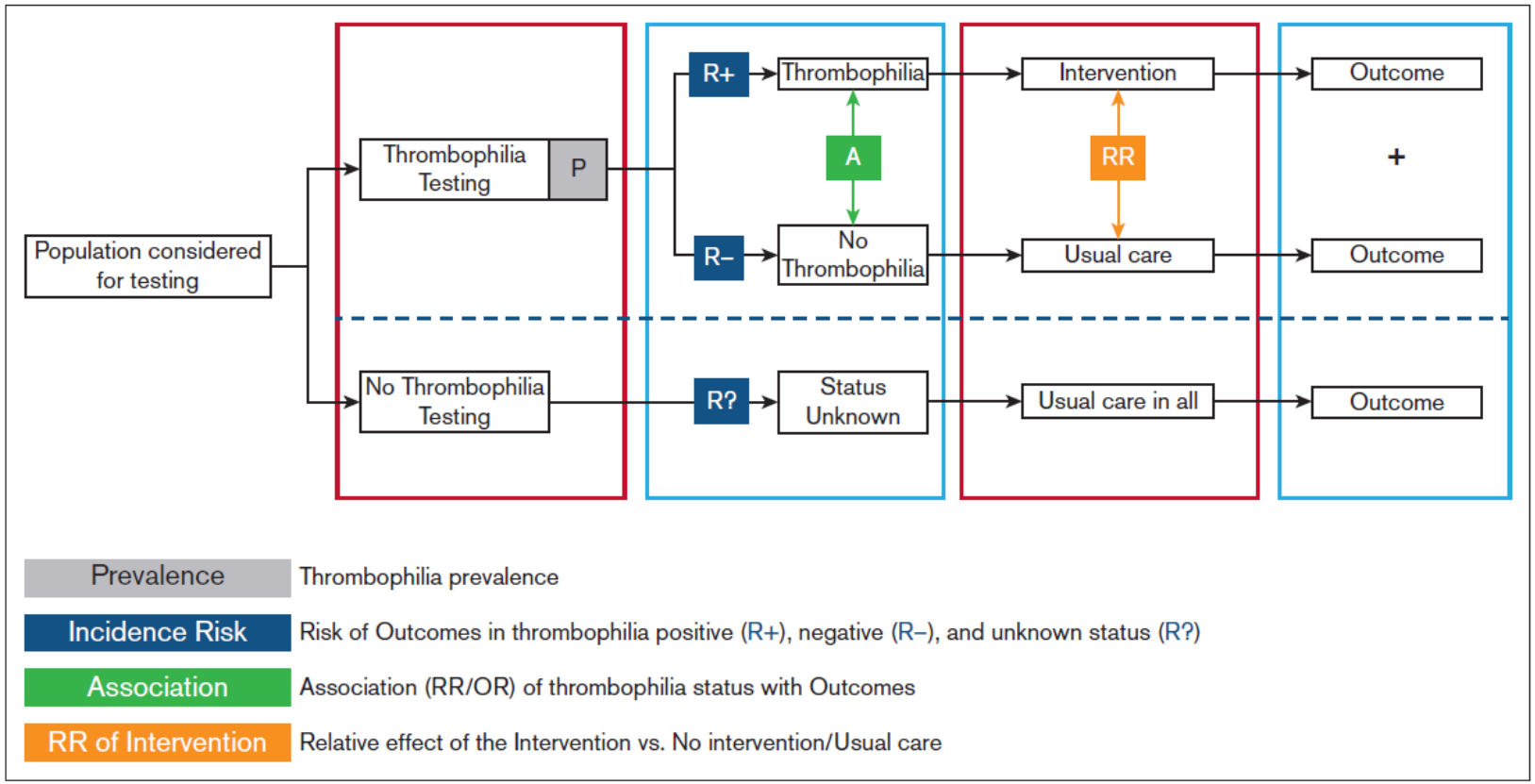


Figure 2. Modeling approach for determining the effect of thrombophilia testing. Population considered for testing: Figure 1 with the guideline flowchart for the different populations for which a recommendation regarding thrombophilia testing was provided. Thrombophilia: any type of thrombophilia or a specific type, depending on whether the recommendation question addresses panel testing or testing for a known specific type in the family. Intervention: course of action other than "usual care." Depending on the specific question, this means prescribing thromboprophylaxis, withholding thromboprophylaxis, extending thromboprophylaxis, stopping thromboprophylaxis, withholding COCs, or withholding HRT. Usual care: for populations where "usual care" was ambiguous, 2 scenarios were modeled, and separate recommendations were provided (see recommendations 7-10).

La trombofilia ereditaria è un esame senza ricadute nelle scelte Terapeutiche : NO

4) To influence VTE secondary prevention

- ✓ In patients with VTE provoked by a nonsurgical major transient risk factor, should thrombophilia testing be performed to guide treatment duration?
- ✓ In women with VTE provoked by pregnancy or postpartum, should thrombophilia testing be performed to guide treatment duration?
- ✓ In women with VTE associated with combined oral contraceptives, should thrombophilia testing be performed to guide treatment duration?

Thrombophilia positive **anticoagulation sine die**
Thrombophilia negative **STOP anticoagulation**

21 fewer recurrence (13 FVL and PT)/1000 pt/year
2-7 more major bleeding/1000 pt/year

Middeldorp S, et al. Blood Adv 2023

Thrombophilia and DOACs

Journal of the American Heart Association

ORIGINAL RESEARCH

Direct Oral Anticoagulants in Patients With Inherited Thrombophilia and Venous Thromboembolism: A Prospective Cohort Study

Elena Campello, MD, PhD; Luca Spiezia, MD, PhD; Chiara Simion, MD; Daniela Tormene, MD, PhD; Giuseppe Camporese, MD; Fabio Dalla Valle, MD, PhD; Anna Poretto, MD; Cristiana Bulato, PhD; Sabrina Gavasso, BS; Claudia Maria Radu, PhD; Paolo Simioni, MD, PhD

Risk of RECURRENCE DURING Anticoagulation =NO DIFFERENCE

Risk of BLEEDING during Anticoagulation =DOAC WORSE

Risk of CRNM BLEEDING during Anticoagulation =DOAC WORSE

Risk of RECURRENCE AFTER STOPPING Anticoagulation =DOAC BETTER

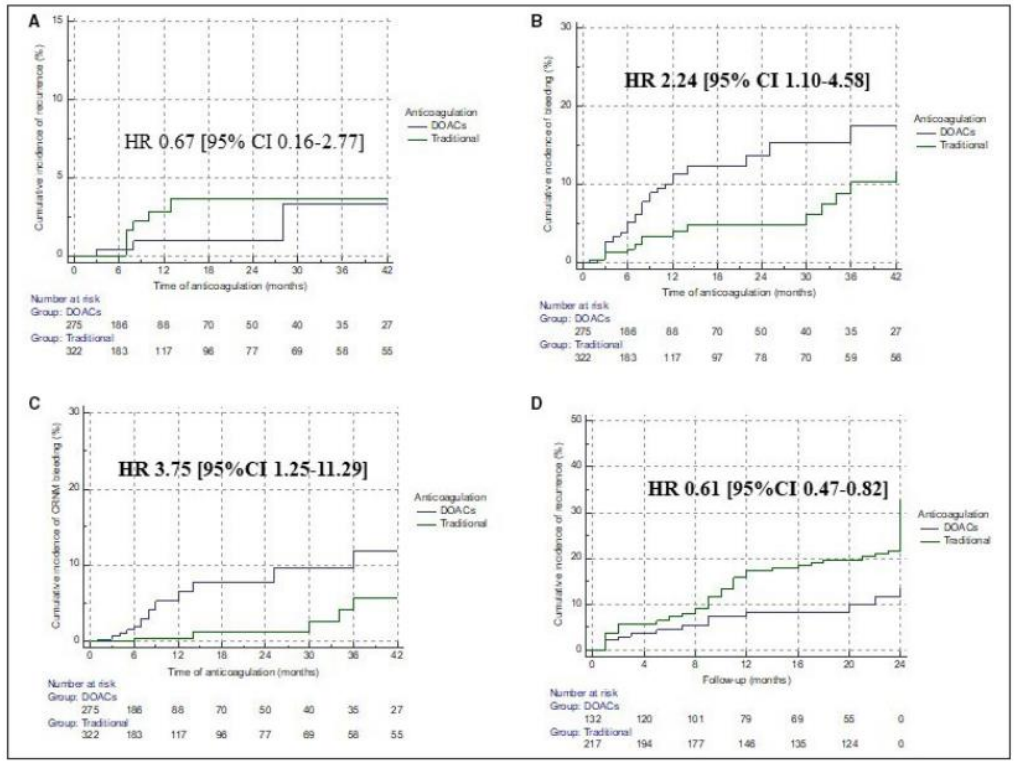


Figure. Cumulative incidence of the study outcomes in patients treated with DOACs vs traditional anticoagulation.
A, Cumulative incidence of recurrent venous thromboembolism during anticoagulation (Log rank test P=0.39). **B**, Cumulative incidence of bleeding during anticoagulation (Log rank test P=0.015). **C**, Cumulative incidence of nonmajor clinically relevant (CRNM) bleeding (Log rank test P=0.0045). **D**, Cumulative incidence of recurrent venous thromboembolism after stopping anticoagulation during 2 years follow-up (Log rank test P=0.0033). DOAC indicates direct oral anticoagulant; and HR, hazard ratio.

J Am Heart Assoc. 2020;9:e018917. DOI: 10.1161/JAHA.120.018917

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

American Heart Association.

Impact of Inherited Thrombophilia on Venous Thromboembolism in Children: A Systematic Review and Meta-Analysis of Observational Studies

Guy Young, Manuela Albisetti, Mariana Bonduel, Leonardo Brandao, Anthony Chan, Frauke Friedrichs, Neil A. Goldenberg, Eric Grabowski, Christine Heller, Janna Journeycake, Gili Kenet, Anne Krümpel, Karin Kurnik, Aaron Lubetsky, Christoph Male, Marilyn Manco-Johnson, Prasad Mathew, Paul Monagle, Heleen van Ommen, Paolo Simioni, Pavel Svirin, Daniela Tormene and Ulrike Nowak-Göttl

ublished online September 8, 2008;

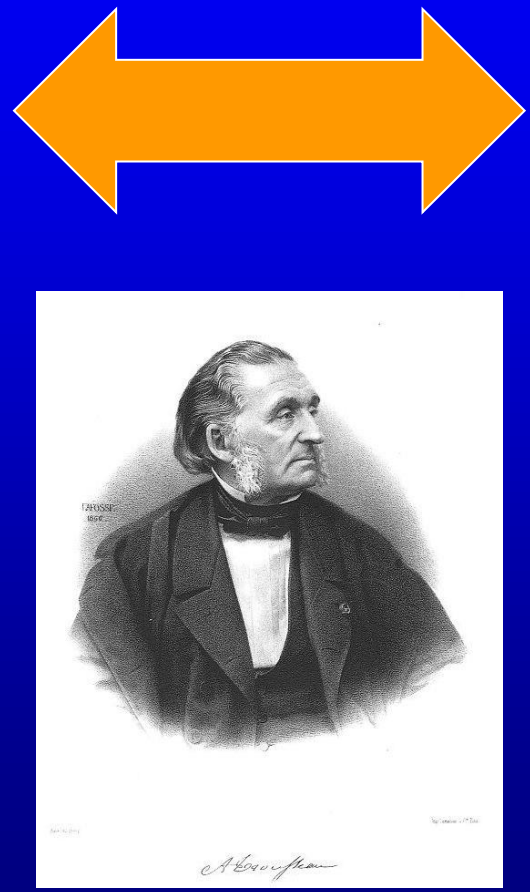
Role of APLA & IT in children with a **first TE onset** * [Odds ratio]

TE-type	Stroke/CSVT	DVT
APLA	6.58	4.87
FV G1691A	3.26	3.55
FII G20210A	2.43	2.64
PC def.	9.31	7.72
PS def.	3.20	5.77
AT def.	7.06	9.44
combined ITs	11.86	9.5

Young et al. Circulation 2008; Kenet et al. Circulation 2010

Cancer & Thrombosis

Cancer



Thrombosis

Armand Trousseau - 1860

THE RISK OF A DIAGNOSIS OF CANCER AFTER PRIMARY DEEP VENOUS THROMBOSIS OR PULMONARY EMBOLISM

HENRIK TOFT SØRENSEN, DR.MED.SCI., LENE MELLEMKJÆR, PH.D., FLEMMING HALD STEFFENSEN, M.D., JØRGEN H. OLSEN, DR.MED.SCI., AND GUNNAR LAUGE NIELSEN, M.D.

APRIL 23, 1998

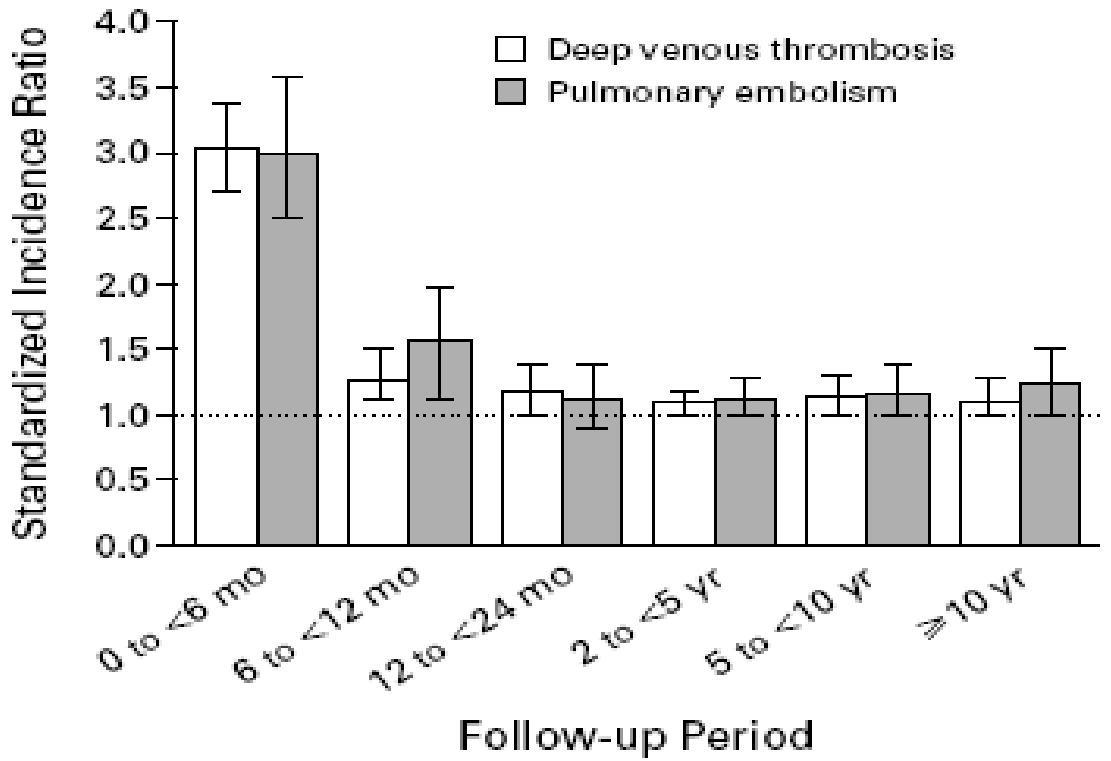


Figure 1. Risk of Cancer in Relation to the Length of the Follow-up Period in 26,653 Patients with Primary Deep Venous Thrombosis or Pulmonary Embolism.

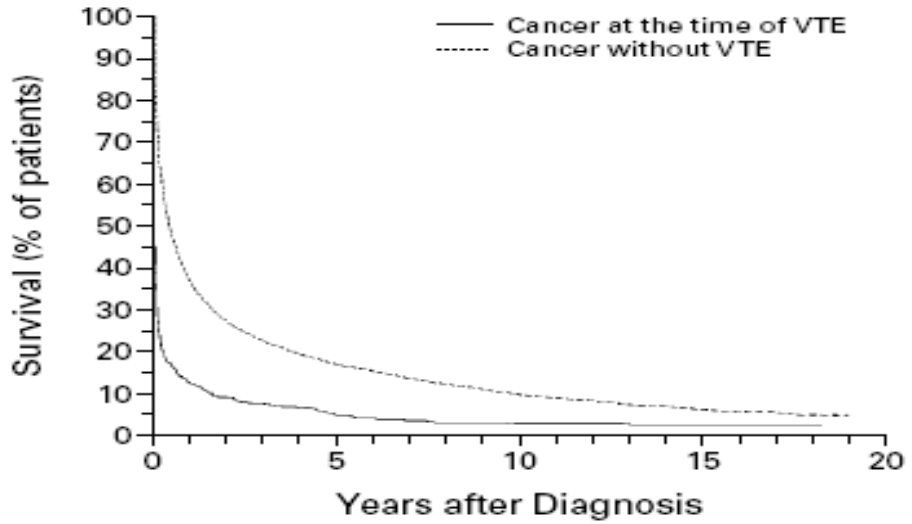
The I bars represent 95 percent confidence intervals.

The New England Journal of Medicine

PROGNOSIS OF CANCERS ASSOCIATED WITH VENOUS THROMBOEMBOLISM

HENRIK TOFT SØRENSEN, DR.MED.SCI., LENE MELLEMKJÆR, PH.D., JØRGEN H. OLSEN, DR.MED.SCI., AND JOHN A. BARON, M.D.

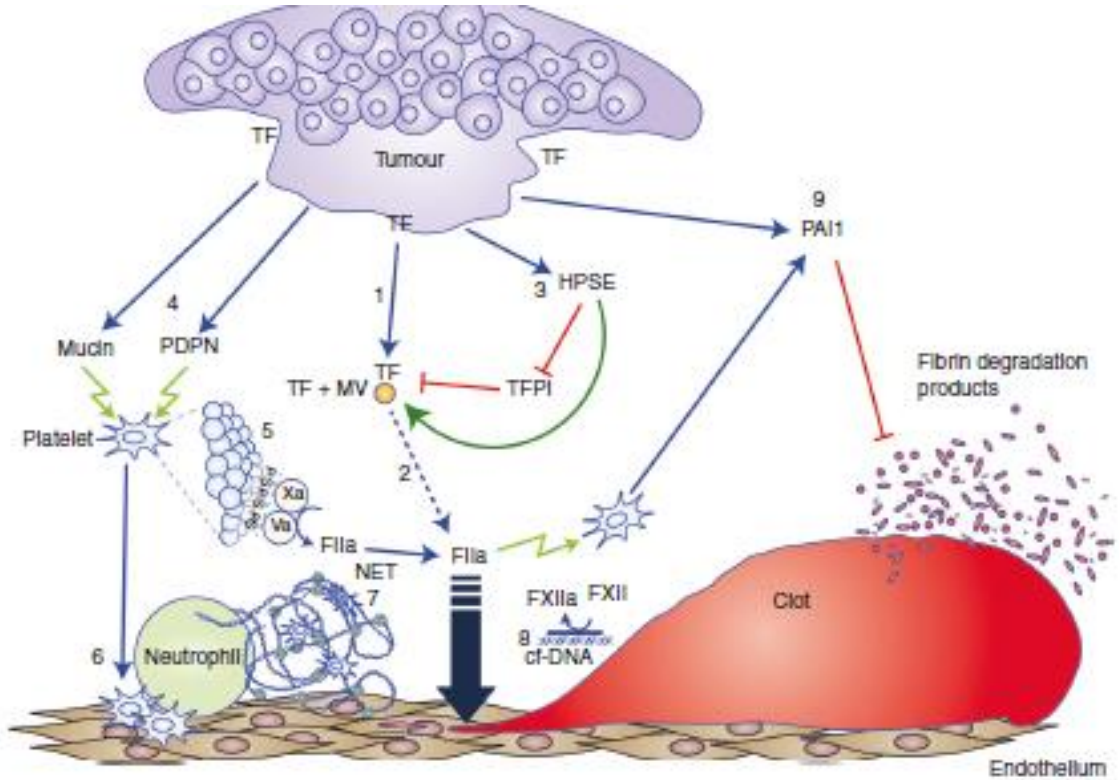
NEJM 2000



No. at Risk				
Cancer at the time of VTE	668	23	10	3
Cancer without VTE	6668	913	338	87

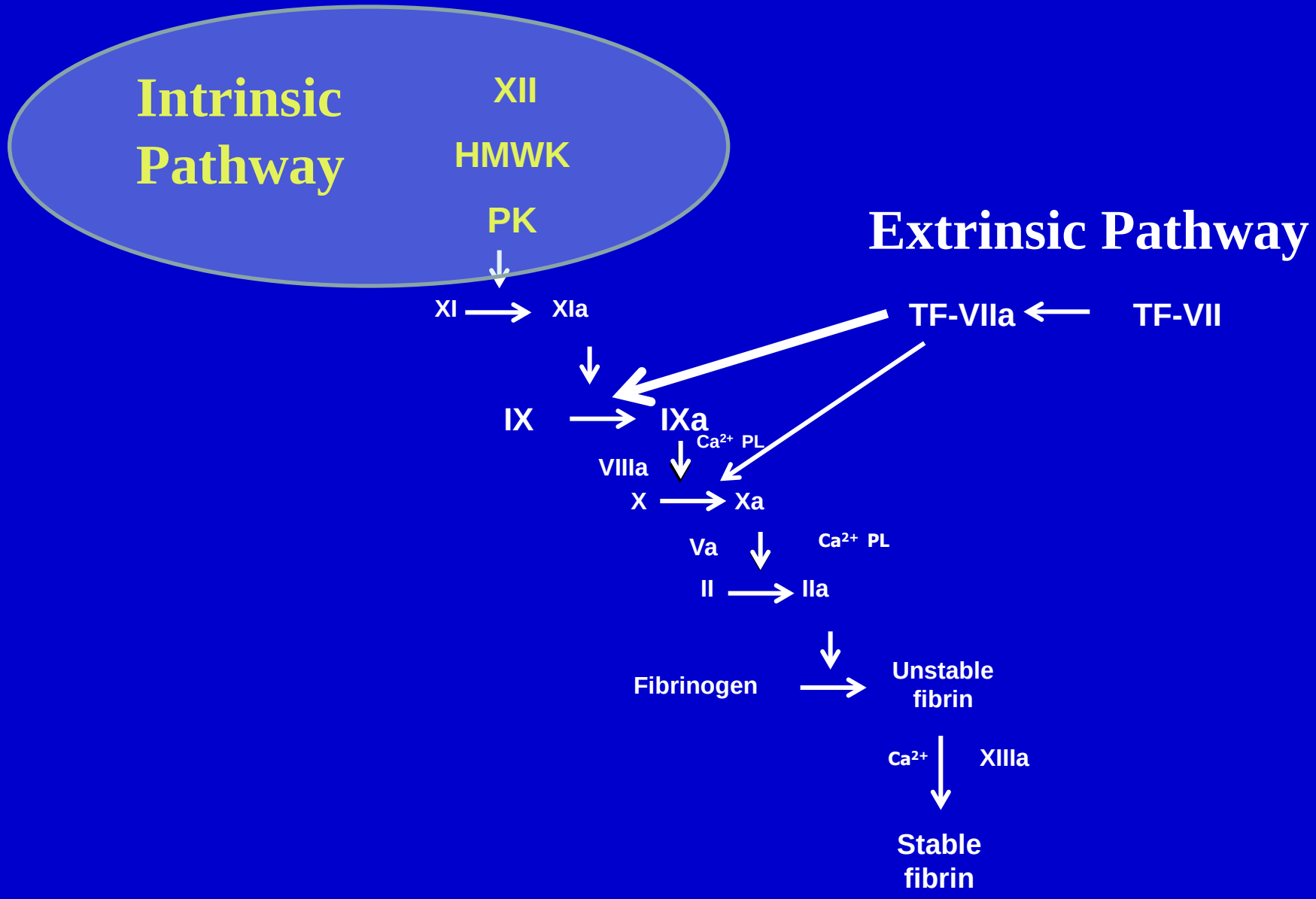
Figure 1. Survival Curves for Patients with a Diagnosis of Cancer at the Time of Venous Thromboembolism (VTE) and Matched Control Patients with Cancer.

The control patients, who did not have venous thromboembolism, were matched with the patients who had venous thromboembolism according to cancer type, sex, age, and year of diagnosis. $P<0.001$ for the overall curves, by the log-rank test.



From Campello et al, BJC 2019

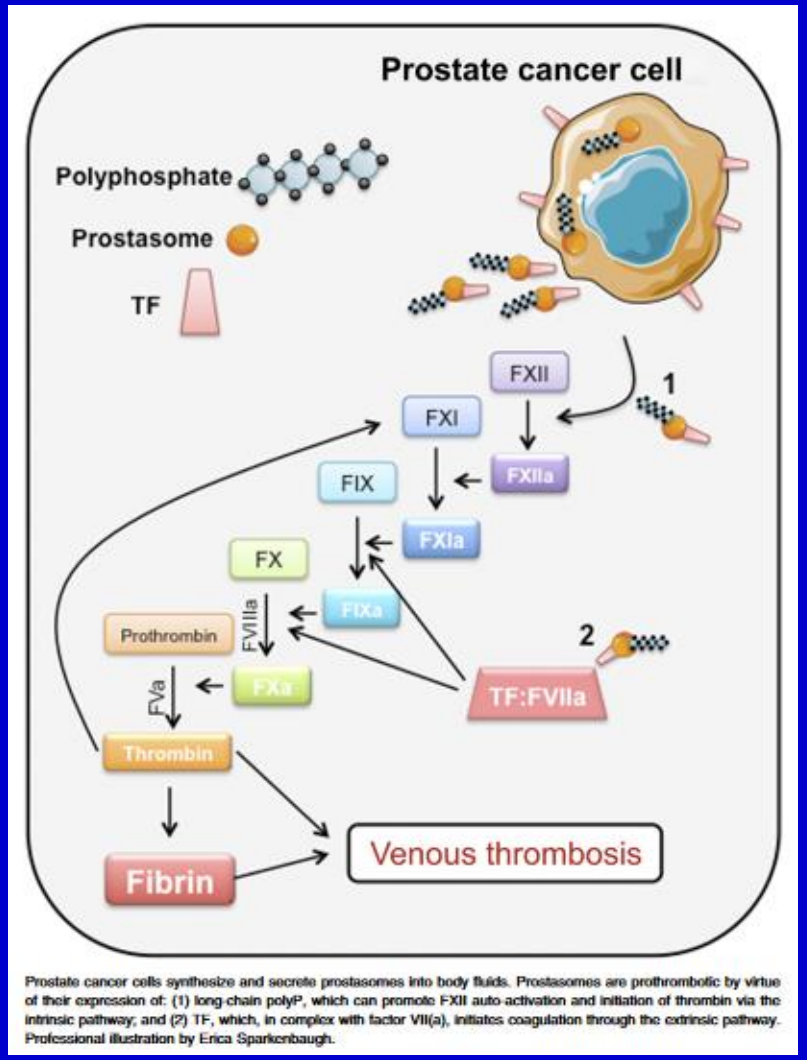
THE CLOTTING "WATERFALL"



New players in Trousseau syndrome

Nigel S. Key UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

In this issue of *Blood*, Nickel et al show that long-chain polyphosphates (polyP) on the surface of secreted microvesicles (MVs) from prostate cancer cells activate coagulation factor XII (FXII), leading to thrombosis.[1]



Prostate cancer cells synthesize and secrete prostasomes into body fluids. Prostasomes are prothrombotic by virtue of their expression of: (1) long-chain polyP, which can promote FXII auto-activation and initiation of thrombin via the intrinsic pathway; and (2) TF, which, in complex with factor VII(a), initiates coagulation through the extrinsic pathway. Professional illustration by Erica Sparkenbaugh.

Blood, 2016

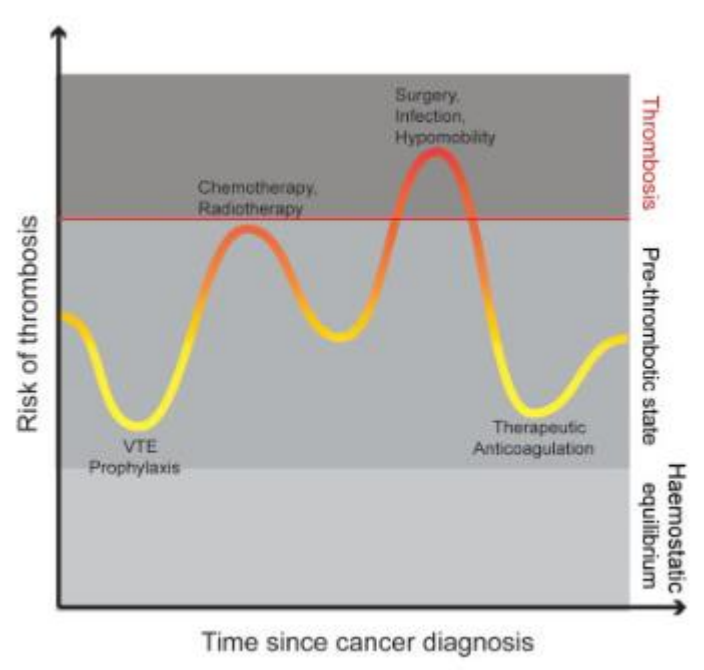
Contact System Activation and Cancer: New Insights in the Pathophysiology of Cancer-Associated Thrombosis

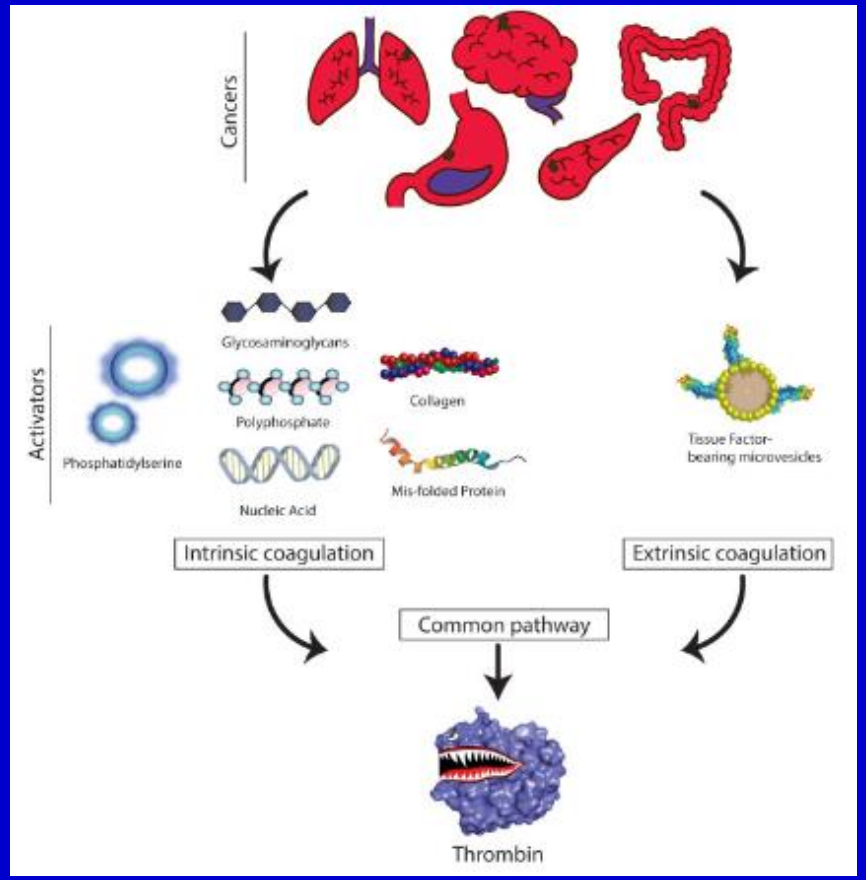
E. Campello[1] M.W. Henderson[2] D.F. Noubouossie[2] P. Simioni[1] N.S. Key[2]

[1] Thrombotic and Hemorrhagic Disease Unit, Department of Medicine, University of Padova, Padova, Italy
[2] Division of Hematology/Oncology, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, United States

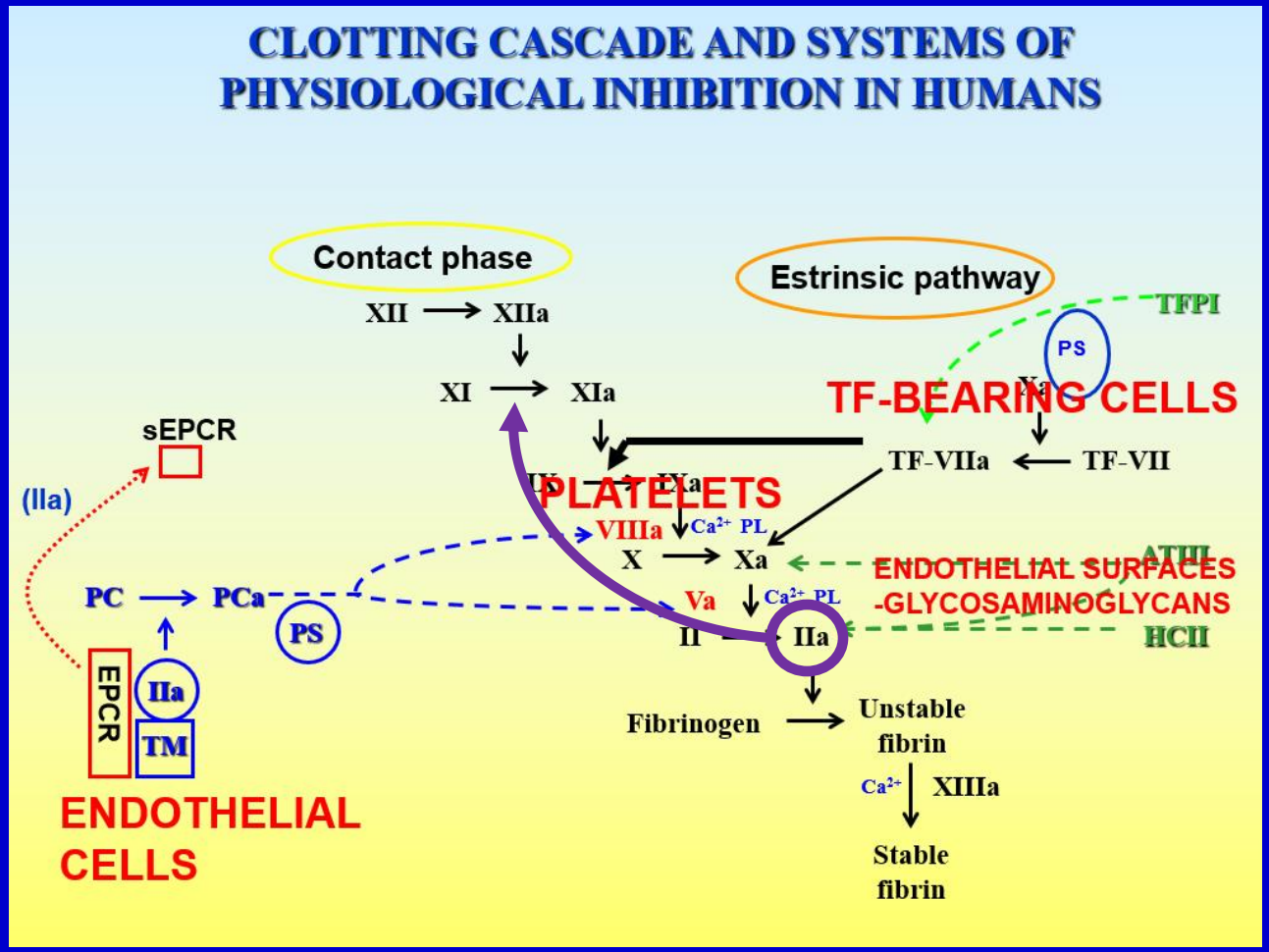
Address for correspondence Nigel S. Key, MB, ChB, FRCP, Division of Hematology/Oncology, Department of Medicine, University of North Carolina at Chapel Hill, 1079 Genetic Medicine Building, CB #7035, 120 Mason Farm Road, Chapel Hill, NC 27599, United States (e-mail: nigel_key@med.unc.edu).

Thromb Haemost 2018;118:251–265.



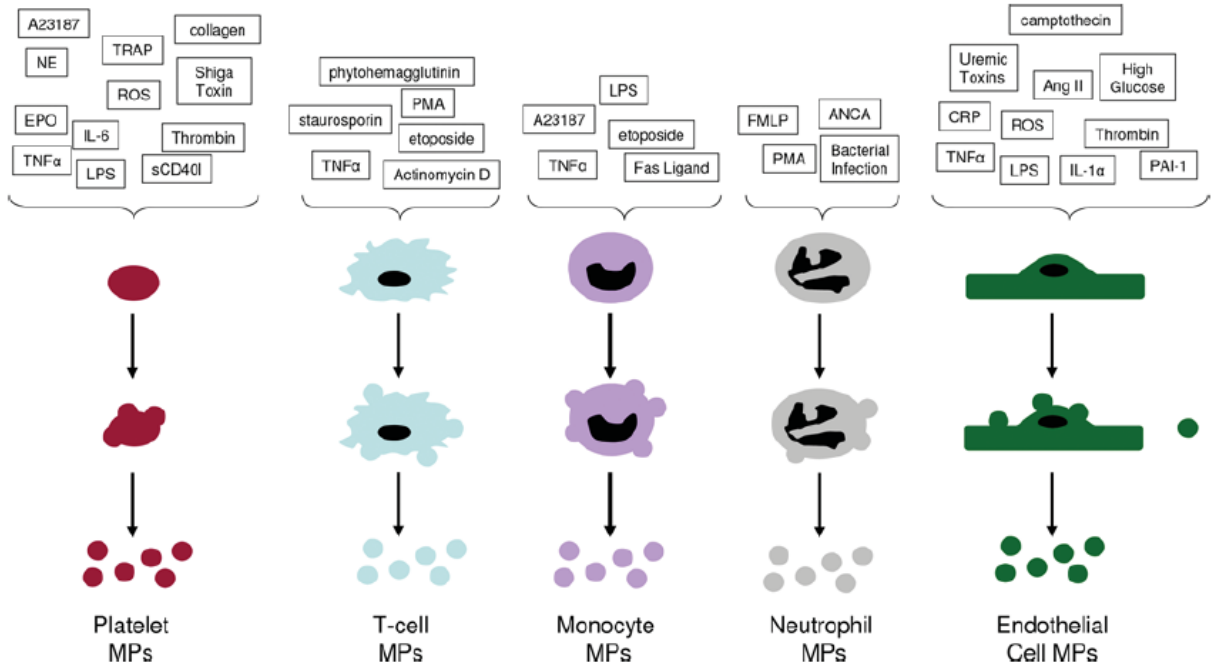


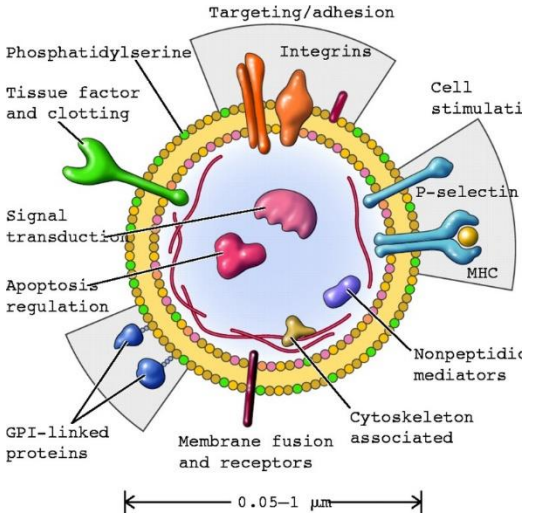
CLOTTING CASCADE AND SYSTEMS OF PHYSIOLOGICAL INHIBITION IN HUMANS



Microparticles – Formation

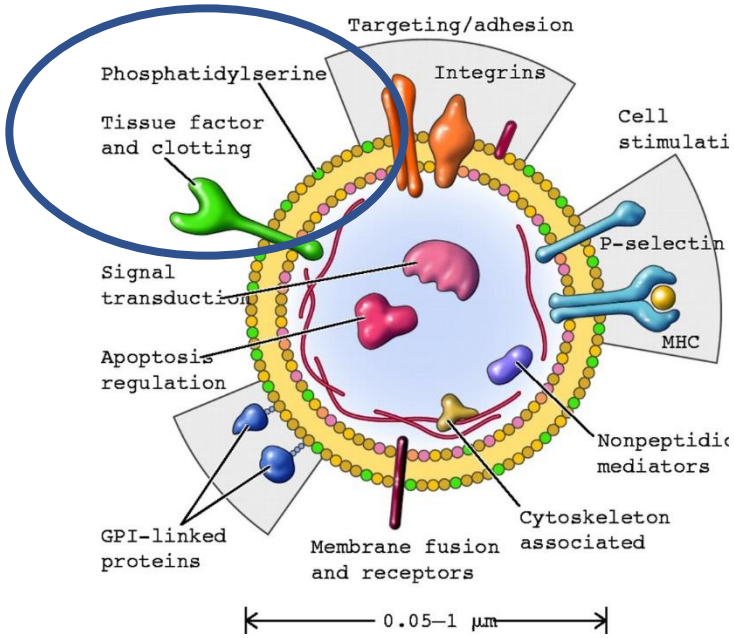
MPs have been predominantly characterized as products of platelets, white blood cells and endothelial cells.

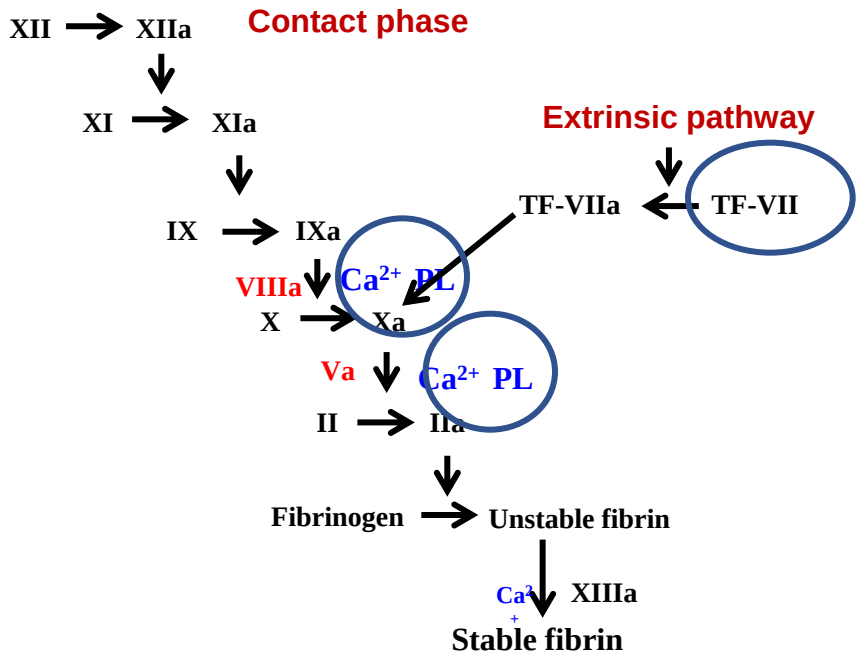




Microparticles – Coagulation

Perhaps the best established property of MPs is their ability to promote coagulation. MPs are elevated in hypercoagulative disorders and this relationship is probably a result of their active participation in the coagulation process.



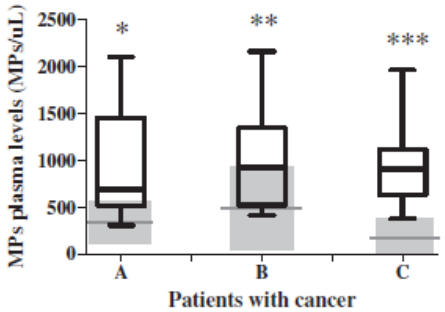


Regular Article

Endothelial, platelet, and tissue factor-bearing microparticles in cancer patients with and without venous thromboembolism

Elena Campello, Luca Spiezia, Claudia M. Radu, Cristiana Bulato, Monica Castelli, Sabrina Gavasso, Paolo Simioni *

Department of Cardiologic, Thoracic, and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Padua, Italy

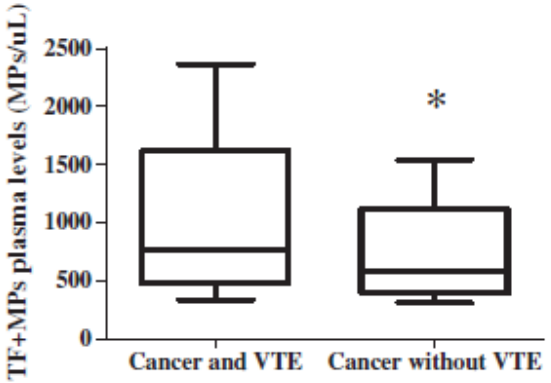


	Case	Controls	p
A	1010 ± 6 39	299 ± 102	<0.001
B	968 ± 482	495 ± 241	<0.001
C	927 ± 415	204 ± 112	<0.001

Platelet-derived MP

Enothelial-derived MP

TF+MP

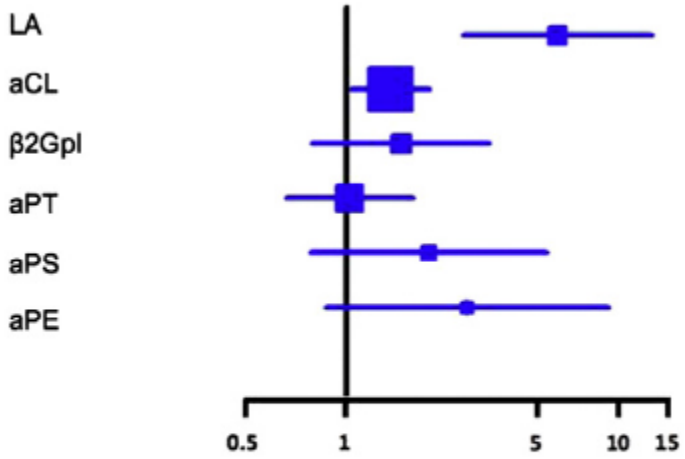


Thrombosis Research 127 (2011) 473–477

Review

Risk of venous and arterial thrombosis according to type of antiphospholipid antibodies in adults without systemic lupus erythematosus: A systematic review and meta-analysis

Quitterie Reynaud [a], Jean-Christophe Lega [b,c,*], Patrick Mismetti [c,d,e], Céline Chapelle [d,f], Denis Wahl [g,h], Pascal Cathébras [a], Silvy Laporte [c,d,f]



	OR [95%CI]	No. studies	No. patients	p ass.	p het.
LA	6.14 [2.74; 13.8]	5	1650	<0.001	0.66
aCL	1.46 [1.06; 2.03]	12	5375	0.02	Random
β2GpI	1.61 [0.76; 3.43]	4	1915	0.22	Random
aPT	1.03 [0.60; 1.77]	3	1639	0.92	Random
aPS	2.03 [0.74; 5.58]	1	276	0.17	Random
aPE	2.82 [0.84; 9.44]	2	474	0.09	Random

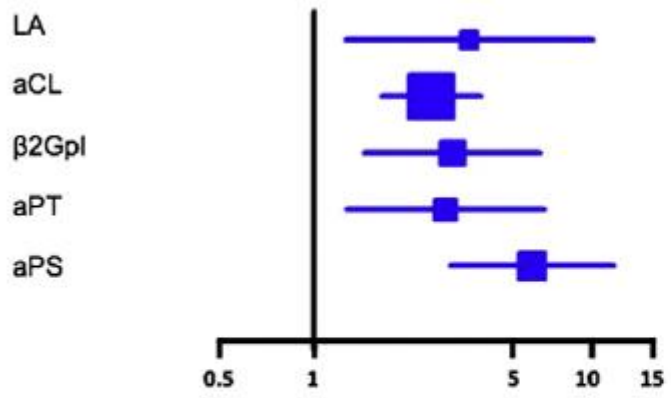
aCL: anti-cardiolipin, aPE: anti-phosphatidyl ethanolamine, aPS: anti-phosphatidyl serine, aPT: anti-prothrombin, LA: lupus anticoagulant, No: number of, OR (95%CI): odds ratio and 95% confidence interval, p ass: p-value for association, p het: p-value for heterogeneity, β2GpI: anti-β2 Glycoprotein I

Fig. 2. Risk of venous thrombosis according to type of antiphospholipid antibody (global forest plot).

Review

Risk of venous and arterial thrombosis according to type of antiphospholipid antibodies in adults without systemic lupus erythematosus: A systematic review and meta-analysis

Quitterie Reynaud [a], Jean-Christophe Lega [b,c,*], Patrick Mismetti [c,d,e], Céline Chapelle [d,f], Denis Wahl [g,h], Pascal Cathébras [a], Silvy Laporte [c,d,f]



	OR [95%CI]	No. studies	No. patients	p ass.	p het.
LA	3.58 [1.29; 9.92]	8	3760	0.01	Random
aCL	2.65 [1.75; 4.00]	17	8289	<0.001	Random
β2GpI	3.12 [1.51; 6.44]	3	1671	0.002	0.44
aPT	2.95 [1.31; 6.66]	2	1327	0.009	0.27
aPS	6.00 [3.07; 11.7]	2	1331	<0.001	0.47

aCL: anti-cardiolipin, aPE: anti-phosphatidyl ethanolamine, aPS: anti-phosphatidyl serine, aPT: anti-prothrombin, LA: lupus anticoagulant, No.: number of, OR (95%CI): odds ratio and 95% confidence interval, p ass: p-value for association, p het: p-value for heterogeneity, β2GpI: anti-β2 Glycoprotein I

Fig. 3. Risk of arterial thrombosis according to type of antiphospholipid antibody (global forest plot).

G.I.R.T.I

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R.I.T.I

REGISTRO ITALIANO TROMBOSI INFANTILE

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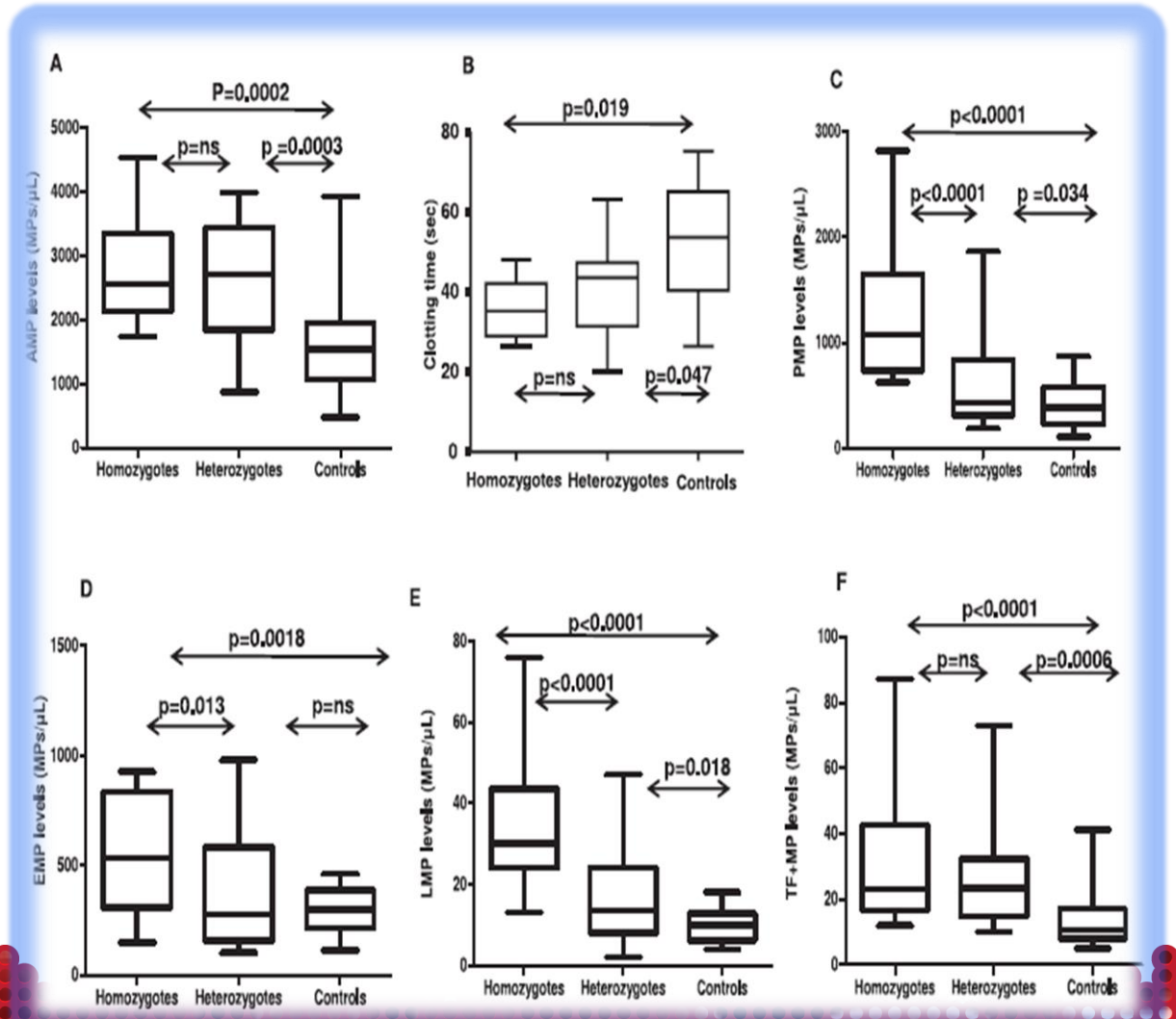
NEW MECHANISMS OF THROMBOSIS?

Circulating microparticles in carriers of factor V Leiden with and without a history of venous thrombosis

Thromb Haemost 2012; 108: 633–639

Elena Campello[1]; Luca Spiezia[1]; Claudia M. Radu[1]; Maria Bon[1]; Sabrina Gavasso[1]; Patrizia Zerbinati[1]; Barry Woodhams[2]; Daniela Tormene[1]; Paolo Prandoni[1]; Paolo Simioni[1]

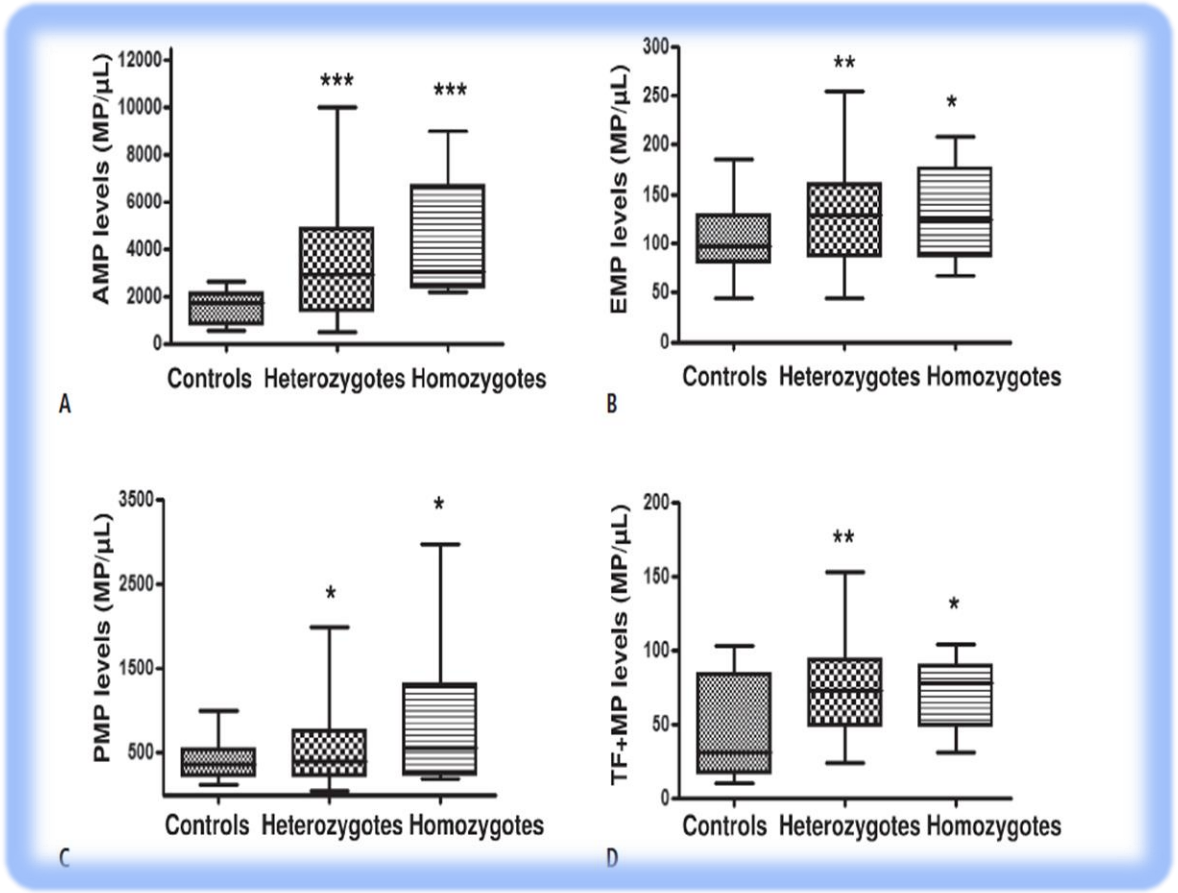
[1]Department of Cardiologic, Thoracic, and Vascular Sciences, 2nd Chair of Internal Medicine, University of Padua Medical School, Padua, Italy; [2]Diagnostica Stago, Gennevilliers, France



Circulating microparticles in carriers of prothrombin G20210A mutation

Elena Campello[1]; Luca Spiezia[1]; Claudia M. Radu[1]; Sabrina Gavasso[1]; Patrizia Zerbinati[1]; Barry Woodhams[1,2]; Paolo Simioni[1]

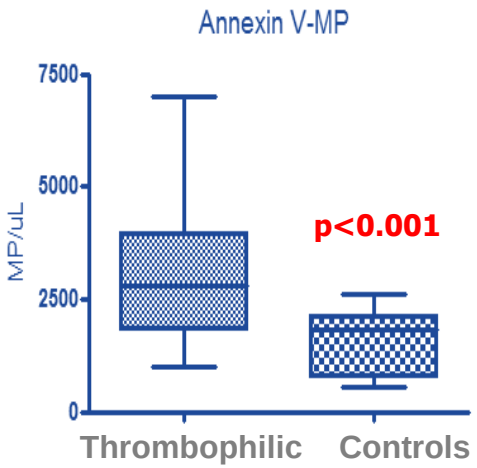
[1]Department of Cardiologic, Thoracic and Vascular Sciences, 2nd Chair of Internal Medicine, Thrombosis and Haemostasis Unit, University of Padua, Italy; [2]HaemaCon Ltd, Bromley, Kent, UK

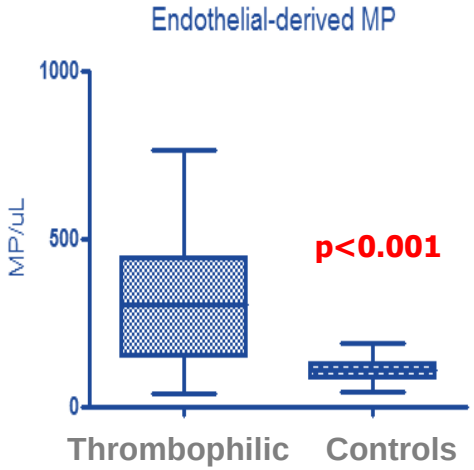


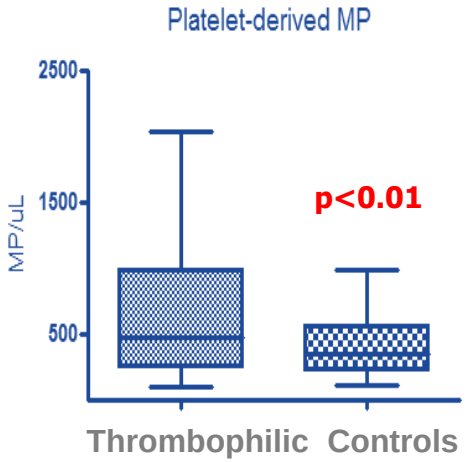
Thromb Haemost 2014; 112: 432–437

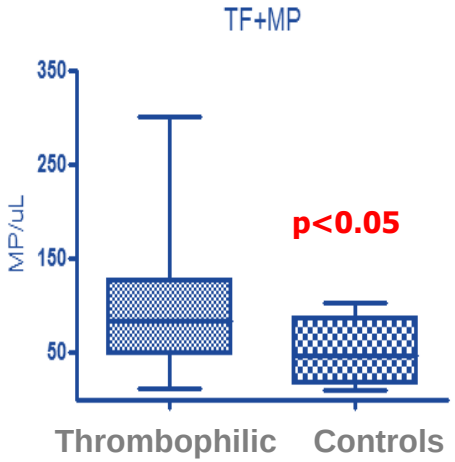
Circulating Microparticles and the risk of thrombosis in inherited deficiencies of antithrombin, protein C and protein S

Elena Campello[1], Luca Spiezia[1], Claudia M. Radu[1], Cristiana Bulato[1], Sabrina Gavasso[1], Daniela Tormene[1], Barry Woodhams[2], Fabio Dalla Valle[1], Paolo Simioni[1].









Thromb & Haemost 2015

Circulating Microparticles and the risk of thrombosis in inherited deficiencies of antithrombin, protein C and protein S

Estimated Odds Ratio for VTE with elevated MP plasma levels

	MP (n/µL) 95 th percentiles	ODD RATIO (95% CI)	
		Univariate	Multivariate
Annexin V-MP	2734	4.01 (2.50-8.34)	3.36 (1.59-7.11)
Endothelial-MP	171	9.26 (3.55-24.1)	7.41 (2.90-15.05)
Platelet-MP	791	3.65 (1.36-8.30)	2.72 (1.16-6.38)
TF+MP	101	1.97 (1.18-3.02)	1.63 (1.02-2.60)

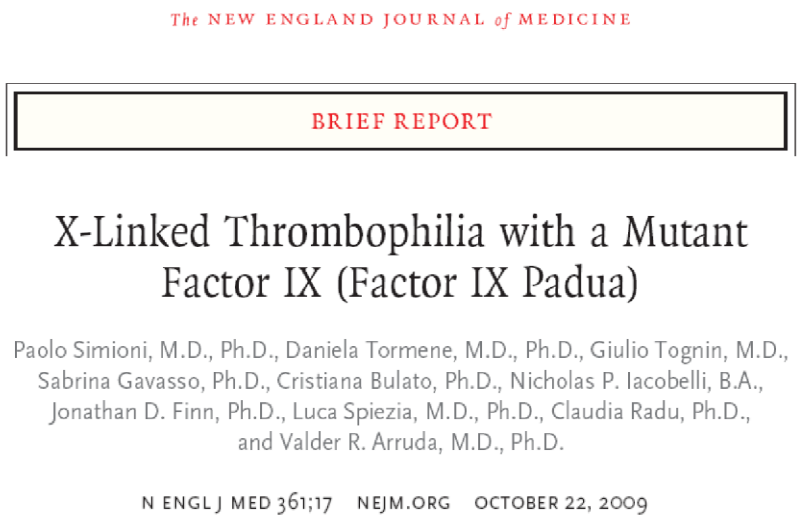
Multivariate analysis was adjusted for age, sex, BMI.

1. UNDIAGNOSED THROMBOPHILIA?

2.NEW INHERITED THROMBOPHILIC CONDITIONS?

Novel hereditary thrombophilia

More recently, sporadic gain-of-function mutations in the genes encoding coagulation factors have been described in individual probands/families with severe thrombophilia.


The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

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

CASE REPORT

- ✓ 23-year-old male
- ✓ Occlusive femoral-popliteal DVT of the right leg
- ✓ (LMWH) (nadroparin, 100U/Kg/twice daily) and warfarin (INR 2.0-3.0)
- ✓ No recurrent thromboembolic event has occurred during the 14-month follow-up
- ✓ Doppler ultrasound one year post-DVT showed partial recanalization of femoral-popliteal veins

Coagulation and thrombophilia screening

- PT, aPTT, fibrinogen, D-Dimer
- Antithrombin, Protein C antigen and activity (chromogenic and amidolytic), Protein S (total, free, activity)
- APC-Resistance and FV Leiden; HR2 Haplotype
- Prothrombin variant G20210A
- FVIII, FIX (antigen and activity), FXI
- Homocysteinemia
- LAC, ACA, anti-beta-2-GPI
- Plasminogen

Hyperfunctional Factor IX (Padua)

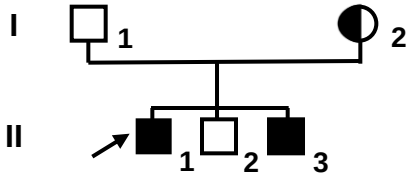
Table 1. Clinical Characteristics and Laboratory Data from the Family Members.*

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	M	53	35.2	105	127	1.2
I-2	F	46	28.2	94	337	3.5
II-2	M	21	33.4	116	123	1.0
II-3	M	11	29.1	64	551	8.6

* II-1 refers to the proband, I-1 to his father, I-2 to his mother, II-2 to the older of his younger brothers, and II-3 to the youngest brother.
† The normal range for activated partial-thromboplastin time is 30 to 40 seconds.

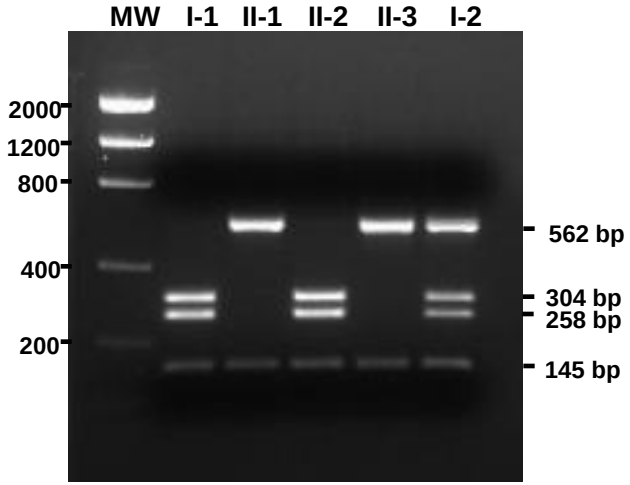
Simioni P et al. NEJM 2009

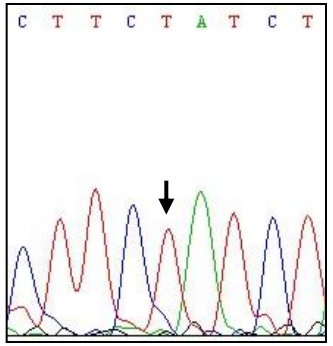
Factor IX PADUA or FIX Arg338Leu (G → T transversion at nt 31134)



Missense mutation at nucleotide 31134

Exon 8 – wild type	CTT	C**G**A	TCT
aa sequence –wild type	337Leu	**338Arg**	339Ser
Exon 8 – **Factor IX Padua**	CTT	C**T**A	TCT
aa sequence – **Factor IX Padua**	337Leu	**338Leu**	339Ser





Proband, II-1

RFLP analysis of exon 8 of the FIX gene digested by TaqI endonuclease.

FIX wild-type → three fragments of 304, 258 and 145 bp. (I-1, II-2)

FIX Padua mutation → two fragments of 562 and 145 bp (II-1, II-3) →hemizygotes

Patients I-2 → four fragments → heterozygous

Simioni P et al. NEJM 2009

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

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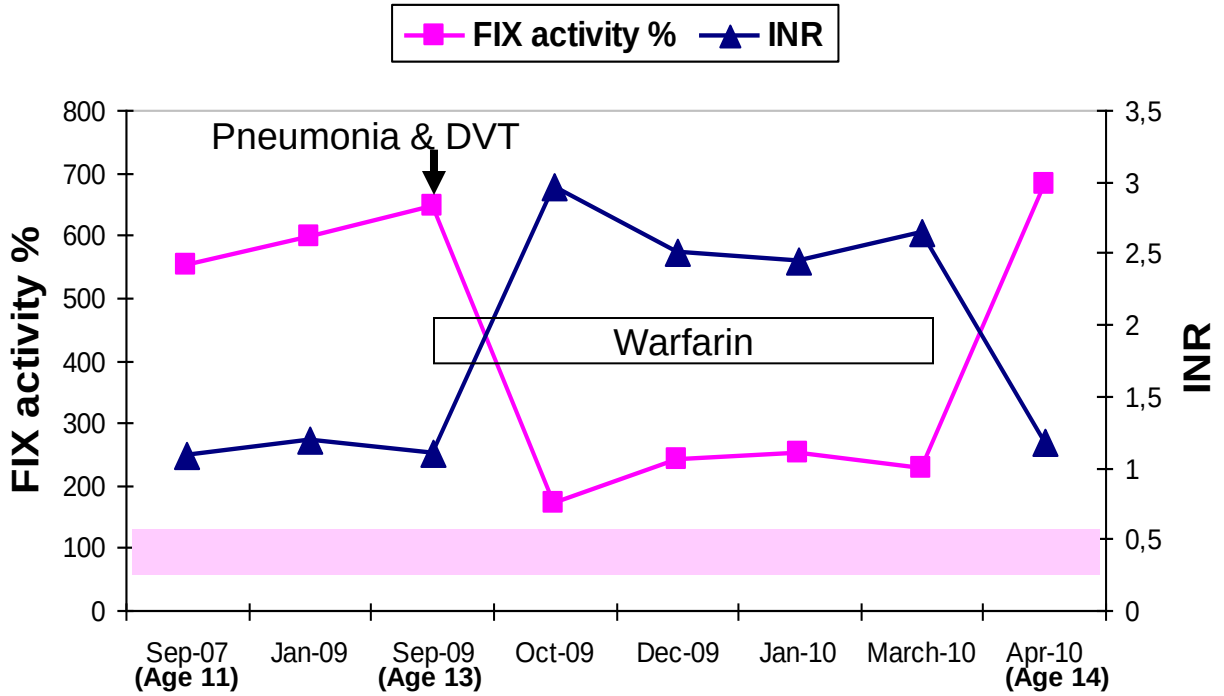
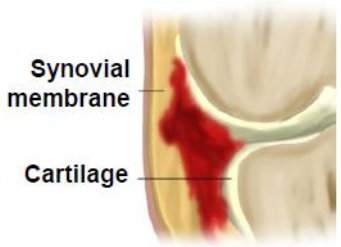
FACTOR IX PADUA AND THROMBOSIS IN CHILDREN

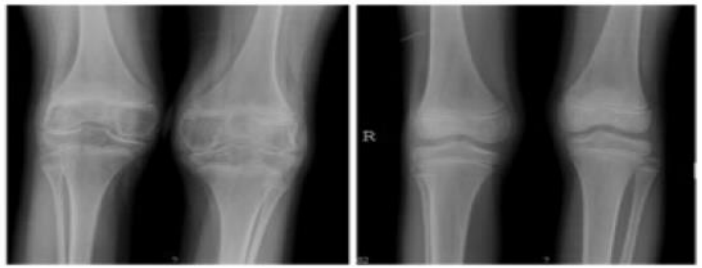


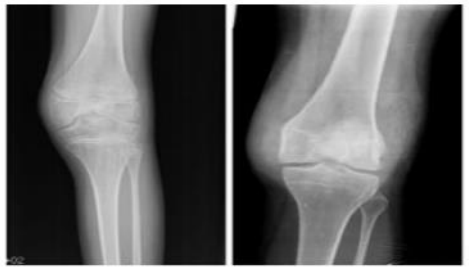
HEMOPHILIA B

Recurrent haemorrhages in the same joint (ie, a target joint) cause:

1. Inflammation of the synovial tissue *(ie, synovitis)*
2. Progressive damage of the tissue
3. Development of arthropathy







Peyvandi F et al. Lancet 2016

canine FIX-Padua

- introduced R338L mutation in canine FIX gene (cFIX-Padua)
- packaged into AAV6 vector
- delivered 3e12 vg/kg via ALP delivery to two HB dogs (M55 and M59)
- 4 weeks immunosuppression

blood

Prepublished online August 23, 2012;
doi:10.1182/blood-2012-06-440123

The efficacy and the risk of immunogenicity of FIX Padua (R338L) in hemophilia B dogs treated by AAV muscle gene therapy

Jonathan D. Finn, Timothy C. Nichols, Nikolaos Svoronos, Elizabeth P. Merricks, Dwight A. Bellenger, Zhangshen Zhou, Paolo Simioni, Katherine A. High and Valder R. Arruda

blood

2012 120: 4517-4520
Prepublished online October 4, 2012;
doi:10.1182/blood-2012-05-432591

Hyperfunctional coagulation factor IX improves the efficacy of gene therapy in hemophilic mice

Alessio Cantore, Nisha Nair, Patrizia Della Valle, Mario Di Matteo, Janka Màtrai, Francesca Sanvito, Chiara Brombin, Clelia Di Serio, Armando D'Angelo, Marinee Chuah, Luigi Naldini and Thierry VandenDriessche

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 DECEMBER 7, 2017 VOL. 377 NO. 23

Hemophilia B Gene Therapy with a High-Specific-Activity Factor IX Variant

L.A. George, S.K. Sullivan, A. Giermasz, J.E.J. Rasko, B.J. Samelson-Jones, J. Ducore, A. Cuker, L.M. Sullivan, S. Majumdar, J. Teitel, C.E. McGuinn, M.V. Ragni, A.Y. Luk, D. Hui, J.F. Wright, Y. Chen, Y. Liu, K. Wachtel, A. Winters, S. Tiefenbacher, V.R. Arruda, J.C.M. van der Loo, O. Zelenaia, D. Takefman, M.E. Carr, L.B. Couto, X.M. Anguela, and K.A. High

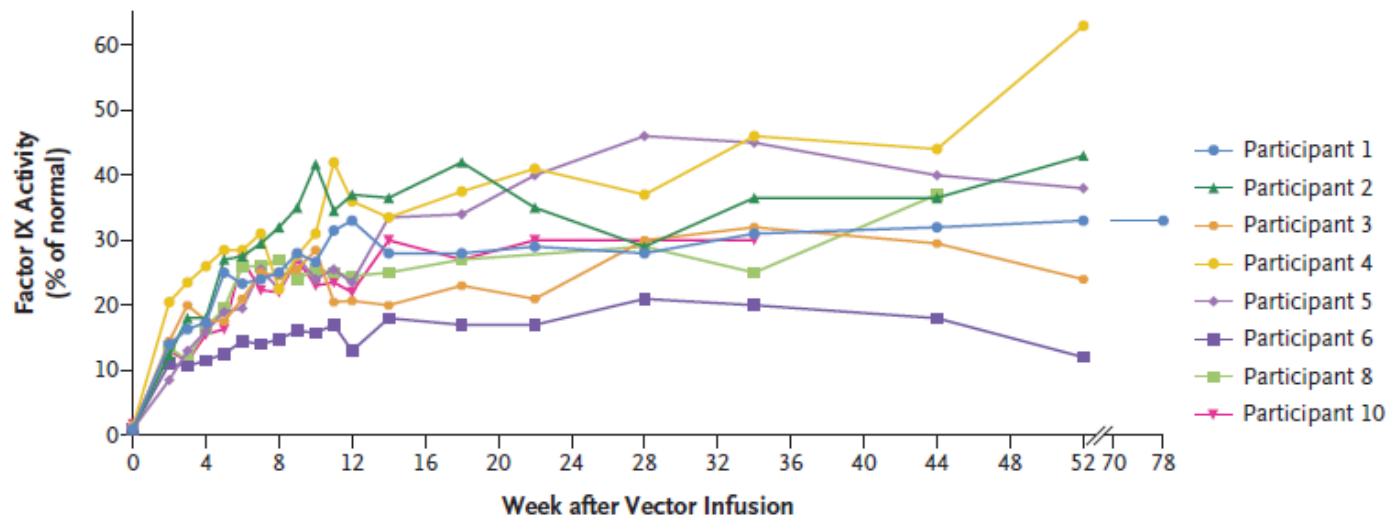


Figure 1. Factor IX Activity after One Peripheral Infusion of SPK-9001 in the Eight Participants Who Did Not Have an Adeno-Associated Viral Capsid-Directed Immune Response.

The vector SPK-9001 was administered at a dose of 5×10^{11} vector genomes per kilogram of body weight.

Hyperactive Factor IX Padua: A Game-Changer for Hemophilia Gene Therapy

Thierry VandenDriessche[1,2] and Marinee K. Chuah[1,2]

https://doi.org/10.1016/j.ymthe.2017.12.007

For the past 25 years, the development of gene therapy for hemophilia has fueled technological innovations and led to emerging insights that benefited the field at large.[1,2] It was particularly encouraging that sustained expression of coagulation factor IX (FIX) was achieved after liver-directed gene therapy with adeno-associated viral vectors (AAVs) in patients with severe hemophilia

the *New England Journal of Medicine*,[4] George and colleagues (Children's Hospital of Philadelphia) and Spark Therapeutics have now achieved this goal and demonstrated sustained FIX coagulant activity of 33.7 ± 18.5% (range, 14% to 81%) after AAV-based gene therapy in all trial participants (9 out of 9) receiving a vector dose of 5×10^{11} vector genomes (vg)/kg.

The improvement in vector performance in this recent clinical trial can be ascribed mainly to the use of a modified FIX transgene encoding a hyperactive mutant FIX protein containing just a single point-mutation (i.e., R338L). This hyperactivating mutation, designated as FIX-R338L-Padua, was initially identified by Dr. Simioni and colleagues[5] (University of Padua) in thrombophilic patients that expressed a FIX protein with an 8-fold increase of specific activity compared to wild-type FIX. Presumably, this R338L mutation results in more efficient thrombin generation. In 2012, we initially demonstrated that the incorporation of this hyperactivating R338L mutation in the FIX gene could augment the efficacy of

[1]Department of Gene Therapy & Regenerative Medicine, Free University of Brussels (VUB), Faculty of Medicine & Pharmacy, Brussels, Belgium; [2]Center for Molecular & Vascular Biology, Department of Cardiovascular Sciences, University

Trials clinici di terapia genica per l'emofilia B

Table 4. Current and Planned Gene Therapy Clinical Trials for Hemophilia B

Sponsor	Treatment	Capsid Serotype	Promoter	Transgene Product	Dose (vg/kg)	Phase	Estimated Enrollment	Status	ClinicalTrials.gov Identifier
Freeline	FLT180a	synthetic	liver-specific	FIX-Padua	6e11–2e12	1	18	recruiting	NCT03369444
					LTFU	2, 3	50	recruiting	NCT03641703
Pfizer	fidanacogene elaparvovec (SPK-9001/PF-06838435)	Spark100	ApoE/hAAT	FIX-Padua	5e11	2	15	active, not recruiting	NCT02484092
					n.d.	3	55	recruiting	NCT03861273
Sangamo	SB-FIX: integration of corrective FIX transgene into albumin locus by AAV6-delivered ZFN	AAV6	–	–	n.d.	1	12	active, not recruiting	NCT02695160
SGIMI	YUVA-GT-F901: autologous HSC/MSC, modified with lentivirus encoding FIX	–	–	–	–	1	10	not yet recruiting	NCT03961243
Takeda	TAK-748 (SHP648/ AskBio009/BAX 335)	scAAV8	TTR	FIX-Padua	2e11–3e12	1, 2	30	active, not recruiting	NCT01687608
SJCRH	scAAV2/8-LP1-FIX	scAAV2/8	LP1	FIX	2e11–2e12	1	14	active, not recruiting	NCT0979238
UniQure	AMT-060	AAV5	liver-specific	FIX	5e12–2e13	1, 2	10	active, not recruiting	NCT02396342
	AMT-061		liver-specific	FIX-Padua	2e13	2	3	recruiting	NCT03489291
						3	56	recruiting	NCT03569891

FIX, clotting factor IX; LTFU, long-term follow-up; n.d., not disclosed; AAV, adeno-associated virus; SGIMI, Shenzhen Geno-Immune Medial Institute; TTR, transthyretin; ApoE/hAAT, apolipoprotein E enhancer/human alpha 1-antitrypsin promoter; ZFN, zinc finger nuclease; SJCRH, St. Jude Children's Research Hospital.

Hyperfunctional Factor IX (Shangai)

Letters to the Editor

Wu W. et al. Haematologica 2021

Factor IX alteration p.Arg338Gln (FIX Shanghai) potentiates FIX clotting activity and causes thrombosis

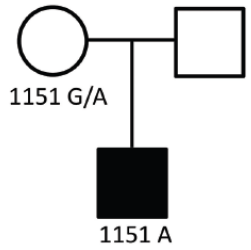
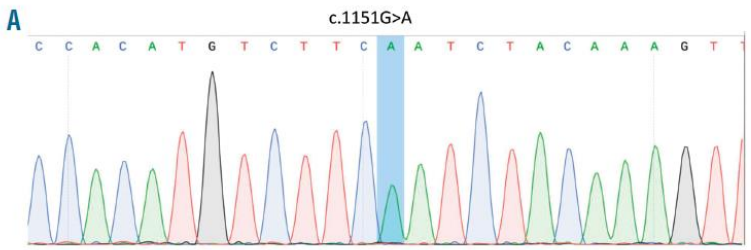
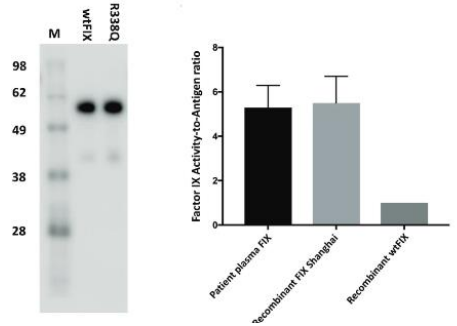


Table 1. The coagulation profile of the patient.

	PT (s)	INR	APTT (s)	Coagulation factor antigen/activity (%) FIX:Ag	FIX:C	FVIII:C	FII:C	FVII:C	FX:C	AT III (%)
Patient	27.3	2.26	38.9	27.5	143.1	126.8	22.5	29.6	10.2	91
Patient's mother	9.3	0.81	29.1	75.8	155.3	155.9	93.6	118.5	96.5	97

PT: prothrombin time; INR: international normalized ratio; APTT: activated partial thromboplastin time; AT III: antithrombin III; F: factor; Ag: antigen; C: coagulation factor activity. Normal reference range for APTT: 25.1~39.5 s; PT: 9.9~15 s; coagulation factor activity: 50~150%; AT III activity: 84.6~120.2%.





BRIEF REPORT

Thrombosis from a Prothrombin Mutation Conveying Antithrombin Resistance

Yuhri Miyawaki, M.Sc., Atsuo Suzuki, M.Sc., Junko Fujita, B.Sc., Asuka Maki, B.Sc., Eriko Okuyama, B.Sc., Moe Murata, B.Sc., Akira Takagi, Ph.D., Takashi Murate, M.D., Ph.D., Shinji Kunishima, Ph.D., Michio Sakai, M.D., Kohji Okamoto, M.D., Ph.D., Tadashi Matsushita, M.D., Ph.D., Tomoki Naoe, M.D., Ph.D., Hidehiko Saito, M.D., Ph.D., and Tetsuhito Kojima, M.D., Ph.D.

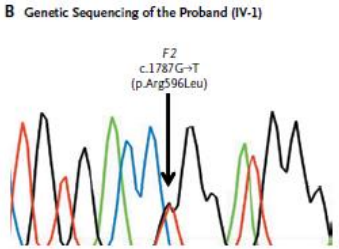
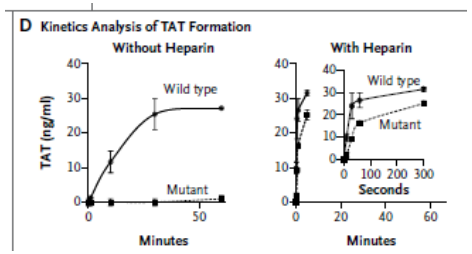


Table 1. Procoagulant and Amidolytic Activities of the Recombinant Prothrombins.*

Prothrombin	Antigen†	Activity‡		
		One-Stage Clotting Assay	Two-Stage Clotting Assay	Chromogenic Assay
		percent		
Wild-type	112	91	109	88
Mutant	118	15	32	66

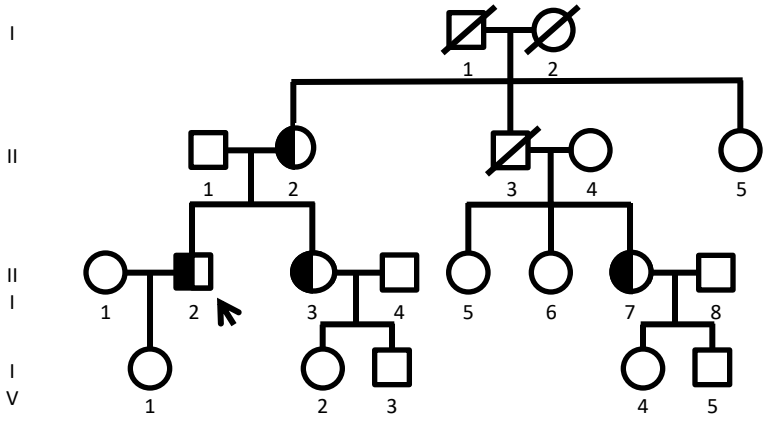


- Novel mechanism of hereditary thrombosis associated with antithrombin resistance, with a substitution of arginine for leucine at position 596 (p.Arg596Leu) in the gene encoding prothrombin.
- The mutant prothrombin had moderately lower activity than wild-type prothrombin in clotting assays, but the formation of thrombin–antithrombin complex was substantially impaired.

N Engl J Med 2012;366:2390-6.

PROTHROMBIN PADUA 2

A) Family 1



Arteriosclerosis, Thrombosis, and Vascular Biology
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American Heart Association.

New Prothrombin Mutation (Arg596Trp, Prothrombin Padua 2) Associated With Venous Thromboembolism

Cristiana Bulato, Claudia Maria Radu, Elena Campello, Sabrina Gavasso, Luca Spiezia, Daniela Tormene and Paolo Simioni

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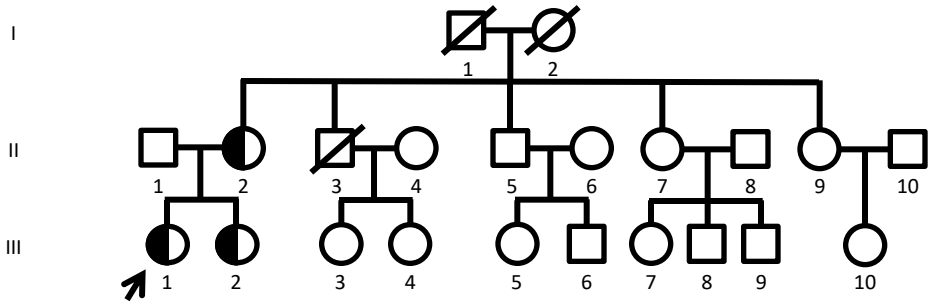
B) Family 2



Bulato, ATVB 2016

Figure 1. Family pedigree
The probands (panel A, III-2 and panel B, III-1) are indicated by an arrow. Open symbols represent normal individuals, while half-filled symbols represent individuals heterozygous for the p.R596W mutation. Squares represent males and circles represent females.

Table 1. Main Clotting Features of Members of the 2 Families Investigated

Family Member	Sex	Age, y	PT, %*†	aPTT, s‡	FII:Ag, %*§	FII:Act, %*§		Phenotype
						PT-Based	ECT-Based	
Family 1								
III-2, proband	M	47	75.5	28.5	80.0	54.0	67.7	Heterozygous
II-2, mother	F	79	91.3	29.6	60.0	58.0	68.4	Heterozygous
III-3, sister	F	53	78.6	32.9	72.0	58.5	66.3	Heterozygous
III-7, cousin	F	55	86.0	31.0	70.0	74.6	80.4	Heterozygous
IV-1, daughter	F	11	84.3	29.7	92.0	99.5	78.7	Normal
Family 2								
III-1, proband	F	29	77.0	28.7	89.0	65.5	63.1	Heterozygous
II-1, father	M	62	107.5	25.2	103.0	117.4	153.8	Normal
II-2, mother	F	58	85.2	30.2	110.0	78.7	83.3	Heterozygous
III-2, sister	F	33	84.8	28.1	120.0	68.2	60.7	Heterozygous

aPTT indicates activated partial thromboplastin time; ECT, ecarin clotting time; FII:Act, prothrombin functional activity; FII:Ag, prothrombin antigen; and PT, prothrombin time.

*PT, FII:Ag, and FII:Act are expressed as the percentage of the normal level.

†The normal range for PT is 70% to 100%.

‡The normal range for aPTT is 24.4–36.5 s.

§The normal range for FII:Ag and FII:Act is 80% to 120%.

Bulato, ATVB 2016

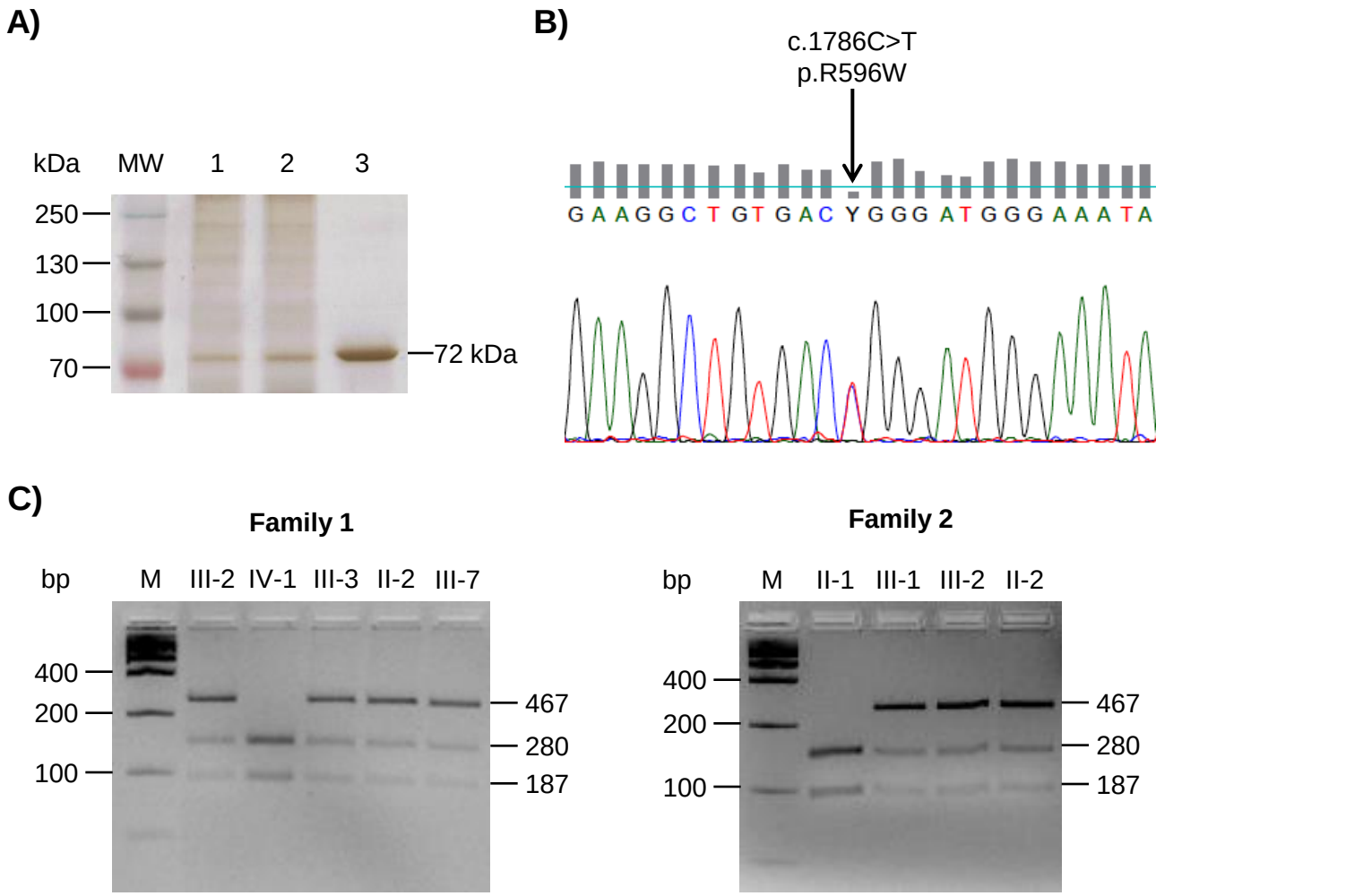


Figure 2. A) SDS-PAGE and silver staining of purified recombinant prothrombins. Line 1. wild type, line 2: mutant (p.R596W), line 3: prothrombin purified from human plasma; MW: molecular weight marker. B) Partial DNA sequence of exon 14 of the prothrombin gene from one proband containing the c.1786C>T (p.R596W) mutation (indicated by an arrow) in heterozygosis. C) *HpaII* digestion of PCR-amplified exon 14 of the two probands and their relatives. Digestion of a normal exon 14 resulted in DNA fragments of 280 and 167 bp. The c.1786C>T mutation abolished the *HpaII* site in exon 14 yielding an additional fragment of 467 bp in the heterozygous patients. In family 1, III-2 refers to the proband, II-2 to his mother, III-3 to his sister, III-7 to his maternal cousin and IV-1 to his daughter. In family 2, III-1 refers to the proband, II-1 to her father, II-2 to her mother and III-2 to her sister. M: DNA ladder.

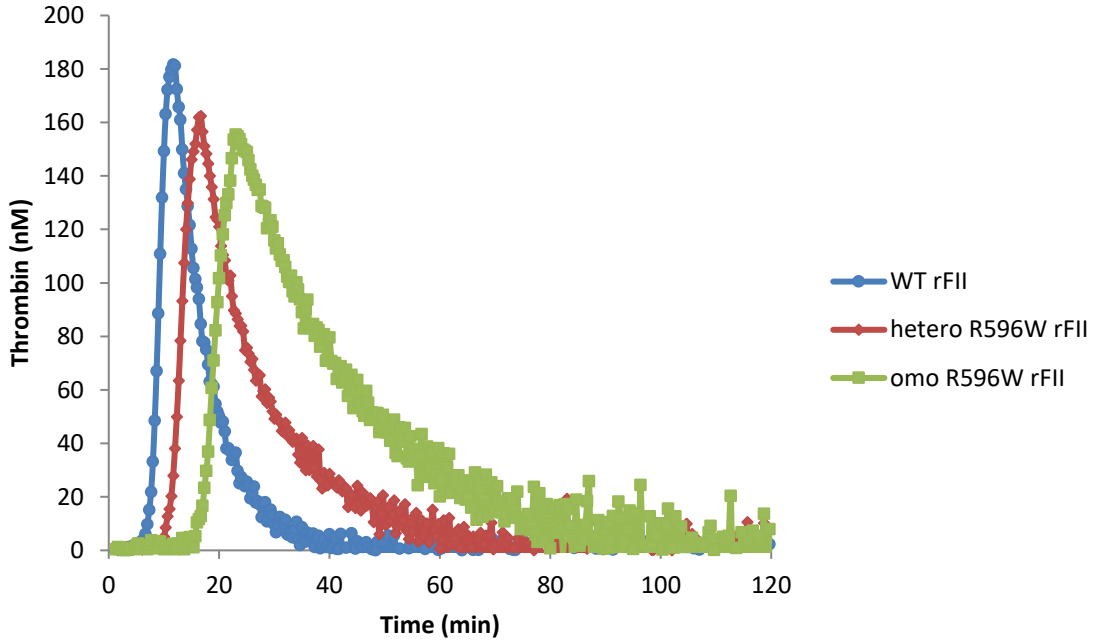
Bulato, ATVB 2016

PROTHROMBOTIC PROTHROMBIN ABNORMALITIES

Table 1 Cases of Prothrombin abnormalities that cause antithrombin resistance and therefore a thrombophilic state

Authors; Year;	Age, Sex	FII act.	FII Ant.	Bleeding	Venous thrombosis (age and first episode)	Mutation	Genotype	Eponym	Comments
Miyawaki et al. (2012)	17, F	37.6 a)	63.8 a)	No	Yes (11 years)	Arg596Leu	Het	Prothrombin Yukuhashi	Patient from Japan
Djordyevic et al (2013)									
Fam 1	n.r., F	n.r	n.r	n.r	Yes (17 years)	Arg596Gln	Het	Prothrombin Belgrade	Six patients in two families
Fam 2	n.r., F	n.r	n.r	n.r	Yes (16 years)	Arg596Gln	Het		
Sivasundar et al. (2013)	60, M	n.r	n.r	n.r	Yes (60 years)	Arg553Gln	Het	Prothrombin Amrita	Patient from India
Kishimoto et al. (2016)	23, F	n.r	n.r	No	Yes, (15 years)	Arg596Gln	Het	Prothrombin Belgrade	Patient from Japan
Bulato et al (2016)									
Fam 1	47, M	54	80	No	Yes (38 years)	Arg596Trp	Het	Prothrombin Padua	Seven patients in two families
Fam 2	29, F	29	89	No	Yes (27 years)	Arg596Trp	Het		

Girolami A. et al. JTT 2017



Measure	Wild type rFII	Hetero R596W rFII	Omo R596W rFII
Lag time (min)	7.67	11.33	17.33
ETP (nM*min)	1711.09	2546.90	3892.63
Peak (nM)	181.60	161.14	154.50
Start tail (min)	44.83	71.00	94.67

Figure 6. Measurement of TG in reconstituted plasmas. TG was assessed with 5 pM TF in prothrombin-deficient plasma reconstituted with the wild type and R596W recombinant prothrombins (wild type rFII and omo R596W rFII, respectively) at a final concentration of 90 μg/ml (100%). Hetero R596W rFII indicates the heterozygous condition, simulated reconstituting prothrombin deficient plasma with 50% wild type recombinant prothrombin and 50% R596W recombinant prothrombin. Table below the TG curves shows the main TG parameters measured.

Bulato, ATVB 2016

Clinical impact of new prothrombin mutations

1. ESTIMATED PREVALENVCE of Prothrombin Padua 2 in patients with VTE is 0.5%

2. Genome-wide linkage scan in thrombophilic families, (GENUT study), ten Kate et al reported a missense mutation in *F2* characterized by the replacement of an Arginine by Tryptophan (the authors did not indicate the amino acid position). Whether this mutation corresponds to prothrombin Padua 2 remains to be defined.

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Regular Article

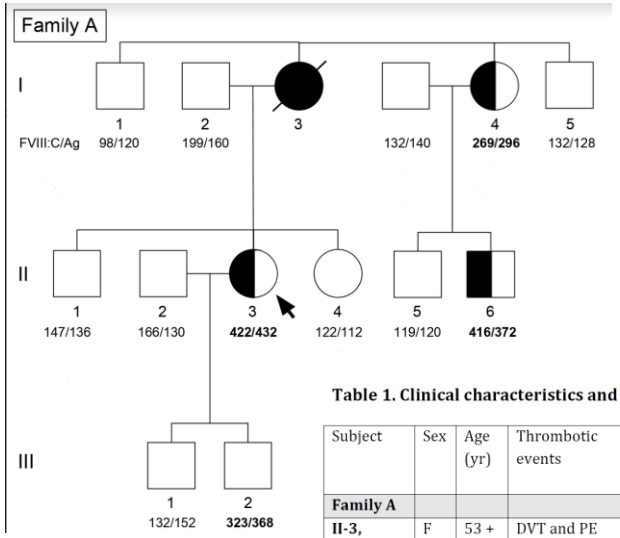
THROMBOSIS AND HEMOSTASIS

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]

[1]General Internal Medicine and Thrombotic and Hemorrhagic Diseases Unit, Department of Medicine, University of Padua Medical School, Padua, Italy; [2]Department of Biology, [3]Centro di Ricerca Interdipartimentale per le Biotecnologie Innovative, and [4]Centro di Miologia, University of Padua, Padua, Italy; [5]Estonian Biocentre, Institute of Genomics, University of Tartu, Tartu, Estonia; and [6]Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands

Blood. 2021;137(17):2383-2393

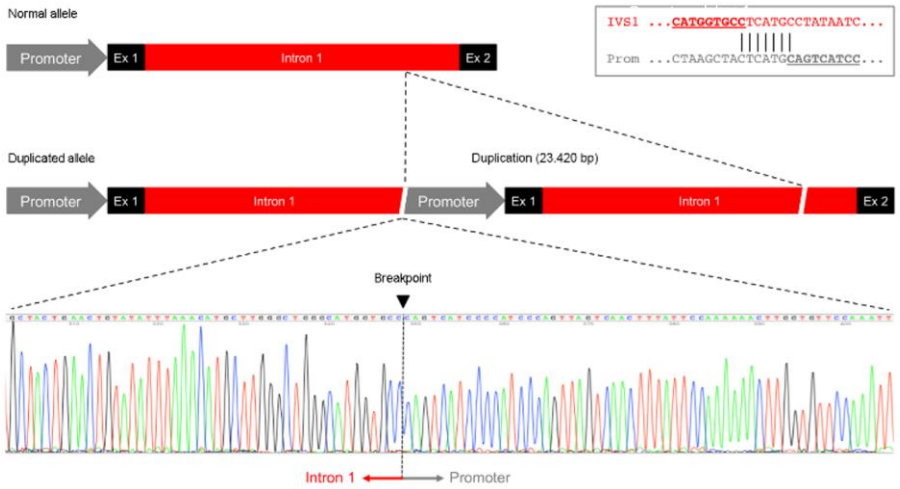


CASE REPORT A

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

Subject	Sex	Age (yr)	Thrombotic events	Age at first VTE (yr)	PT -INR	aPTT (sec) (normal range: 26-34 sec)	FVIII:C (%) (normal range: 58-162%)	FVIII:Ag (%) (normal range: 64-156%)	VWF: Ag (%) (normal range: 52-178%)	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

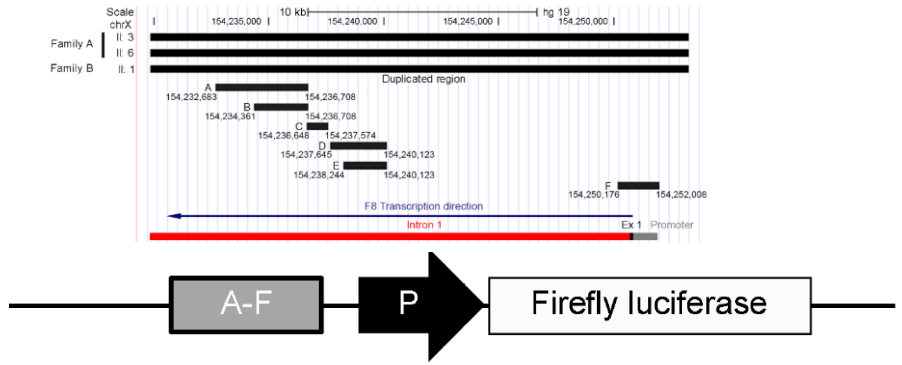
F8 duplication and breakpoint mapping



The duplication spans 23,420 bp (GRCh37 154,229,849-154,253,268) and includes the promoter, exon 1 and nearly the whole intron 1 of the F8 gene.
The two copies are arranged in tandem without spacer DNA and have the same orientation

Simioni P. et al. Blood 2021 in press

Functional analysis of the *F8* duplication



Highest transcriptional activity (>45-fold enhancement) was associated with region C, followed by region F (~20-fold enhancement), which contains the *F8* proximal promoter.

Simioni P. et al. Blood 2021

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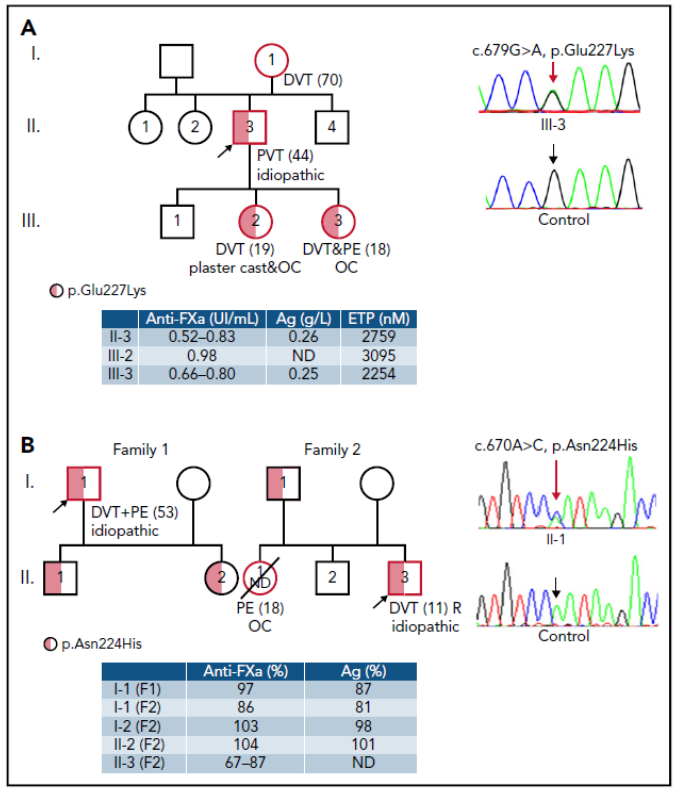
THROMBOSIS AND HEMOSTASIS

Two *SERPINC1* variants affecting N-glycosylation of Asn224 cause severe thrombophilia not detected by functional assays

Maria Eugenia de la Morena-Barrio,[1] Pierre Suchon,[2] Eva Marie Jacobsen,[3] Nina Iversen,[4] Antonia Miñano,[1] Belén de la Morena-Barrio,[1] Carlos Bravo-Pérez,[1] Jose Padilla,[1] Rosa Cifuentes,[1] Susana Asenjo,[5] Jean François Deleuze,[6-8] David Alexandre Trégouët,[6-9] Maria Luisa Lozano,[1] Vicente Vicente,[1] Per Morten Sandset,[3] Pierre Emmanuel Morange,[2] and Javier Corral[1]

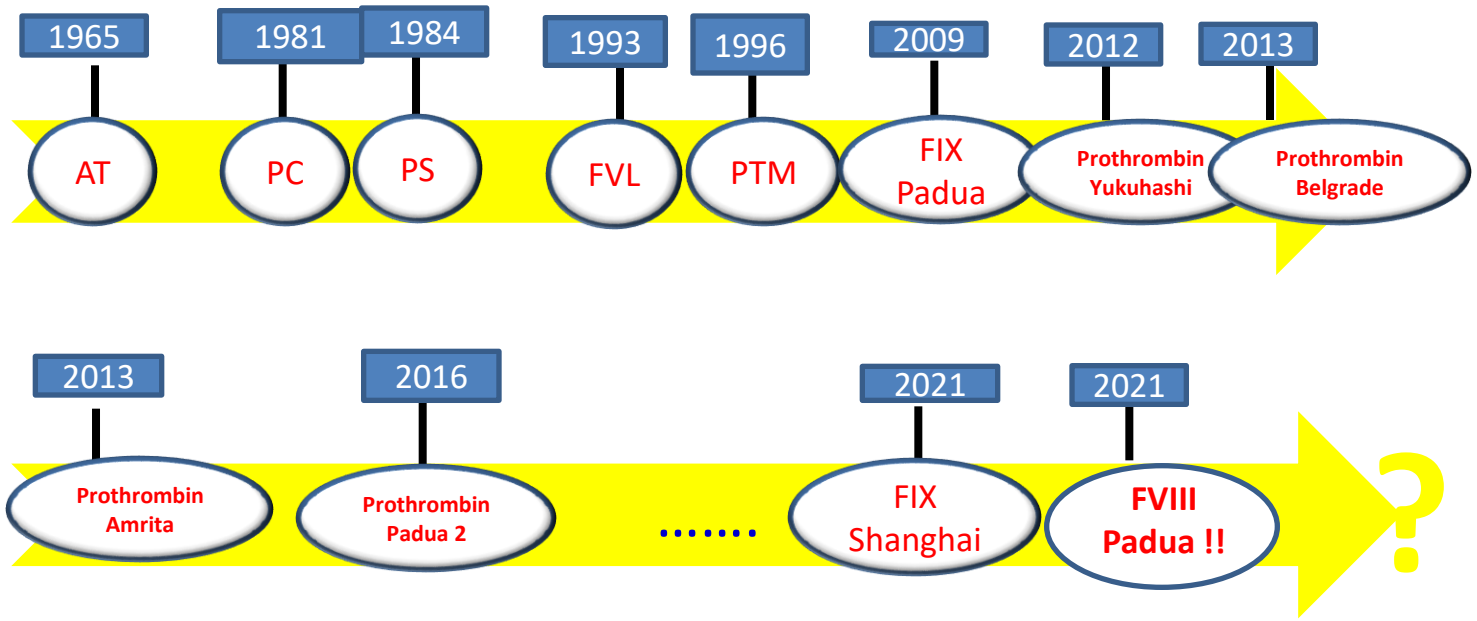


	Anti-FXa (UI/mL)	Ag (g/L)	ETP (nM)
II-3	0.52–0.83	0.26	2759
III-2	0.98	ND	3095
III-3	0.66–0.80	0.25	2254

	Anti-FXa (%)	Ag (%)
I-1 (F1)	97	87
I-1 (F2)	86	81
I-2 (F2)	103	98
II-2 (F2)	104	101
II-3 (F2)	67–87	ND

Figure 1. Thrombophilic families carrying new *SERPINC1* variants affecting *N*-glycosylation of Asn224. Clinical information, including type and age of the first thrombotic event (between brackets) and recurrence, functional values (anti-FXa activity), antigen levels, and molecular data (including the electropherogram of exon 4 in a symptomatic patient and a healthy control patient) are shown. Symbols half filled with red represent heterozygous subjects, and a red border indicates a patient who had a thrombotic event. The proband is pointed by an arrow. (A) p.Glu227Lys. Pedigree tree of the index French thrombophilic family. Thrombin generation data in available subjects are also shown. The c.679G>A (p.Glu227Lys) mutation is pointed by a red arrow. (B) p.Asn224His. Pedigree 3 of 2 unrelated Norwegian families. The c.670A>C (p.Asn224His) mutation is pointed by a red arrow. DVT, deep venous thrombosis; PVT, portal venous thrombosis; PE, pulmonary embolism; ND, not determined; AT Ag, antithrombin antigen; ETP, endogenous thrombin potential; OC, oral contraceptives; R, recurrence; F1, family 1; F2, family 2.

In conclusion, we identified 2 new SERPINC1 defects that cause hypoglycosylation of Asn224. These variants have minor, if any, functional consequences when using routine methods to diagnose antithrombin deficiency but increase thrombin generation
and reduce the inhibition of FVIIa.


1965
AT
1981
PC
1984
PS
1993
FVL
1996
PTM
2009
FIX Padua
2012
Prothrombin Yukuhashi
2013
Prothrombin Belgrade
2013
Prothrombin Amrita
2016
Prothrombin Padua 2
.....
2021
FIX Shanghai
2021
FVIII Padua !!
?

EXPERT REVIEW OF HEMATOLOGY
https://doi.org/10.1080/17474086.2020.1804354

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REVIEW

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Genetic risk factors for venous thromboembolism

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Figure 2. Confirmed genes linked to venous thromboembolism (VTE) in family studies [23–25] or genome wide association studies [43,44] grouped according to potential relation to VTE, i.e. anticoagulation, coagulation, platelets, erythrocytes, immune system, and unknown.

Next Generation Sequencing -NGS

Antithrombin defect …ONLY??

E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
Allele	Reference allele	Length	Zygosity	Count	Coverage	Frequency		ENSEMBL	ENSEMBL (Homo_sapiens_ensembl_v87_hg19_mRNA)	Coding region change	Amino acid change	Amino acid change in longest transcript	Coding region change in longest transcript	Gene Name	dbSNP	
C	No	1	Heterozyg	318	657	48,4	F5	ENS	ENST000(	ENST000(	ENSP000(	ENSP00000356770:p.Asp2227Gly	ENST00000367796:c.6680A>G	F5, F5, F5	6027	benigna
C	No	1	Heterozyg	381	818	46,7	F5	ENS	ENST000(	ENST000(	ENSP000(	ENSP00000356770:p.Met1769Val	ENST00000367796:c.5305A>G	F5, F5	6030	benigna
G	No	1	Heterozyg	498	1013	49,1	F5	ENS	ENST000(	ENST000(	ENSP000(	ENSP00000356770:p.Asn822Thr	ENST00000367796:c.2465A>C	F5, F5	6018	benigna
C	No	1	Heterozyg	403	932	4	F5	ENS	ENST000(	ENST000(	ENSP000(	ENSP00000356770:p.Gln534Arg	ENST00000367796:c.1601A>G	F5, F5, F5	6025	benigna
T	No	1	Heterozyg	1291	2729	,30670	SERPINC1	ENSG0	ENST000(	ENST000(	ENSP000(	ENSP00000356671:p.Glu227Lys	ENST00000367698:c.679G>A	SERPINC1, SERPINC1		VUS
T	No	1	Homozyg	435	435	100	F13B	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000356382:p.Arg115His	ENST00000367412:c.344G>A	F13B	6003	benigna
C	No	1	Heterozyg	328	670	8,95522	TFPI, AC007319.1	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000342306:p.Asn221Ser; ENSP00000386344:p.Asn221S	ENST00000233156:c.628+5354A>G	AC007319.1, AC007319.1,	7586970	benigna
C	No	1	Homozyg	2296	2298	99,91296	KNG1, RP11-573D15.8	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000265023:p.Met178Thr	ENST00000265023:c.533T>C	RP11-573D15.8, KNG1, KN	1656922	benigna
C	No	1	Homozyg	2251	2254	99,86690	KNG1, RP11-573D15.8	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000265023:p.Ile581Thr	ENST00000265023:c.1742T>C	RP11-573D15.8, KNG1, KN	710446	benigna
C	No	1	Heterozyg	416	887	46,89966	FGA	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000306361:p.Thr331Ala	ENST00000302053:c.991A>G	FGA, FGA	6050	benigna
C	No	1	Heterozyg	597	1189	50,21026	KLKB1	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000424469:p.Trp541Arg	ENST00000264690:c.1761T>C	KLKB1, KLKB1, KLKB1, KLK	925453	benigna
G	No	1	Homozyg	1212	1212	100	F12, GRK6	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000253496:p.Ala207Pro	ENST00000253496:c.619G>C	F12, GRK6, F12, GRK6	17876030	benigna
A	No	1	Heterozyg	628	1265	49,64426	F13A1	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000264870:p.Val35Leu	ENST00000264870:c.103G>T	F13A1, F13A1, F13A1, F13	5985	benigna
A	No	1	Heterozyg	263	509	51,66994	PLG	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000308938:p.Asp472Asn	ENST00000308192:c.1414G>A	PLG, PLG, PLG	4252125	benigna
C	No	1	Homozyg	2533	2535	99,92110	VWF	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000261405:p.Gln852Arg	ENST00000261405:c.2555A>G	VWF, VWF	216321	benigna
C	No	1	Homozyg	2647	2652	99,81146	VWF	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000261405:p.Thr789Ala	ENST00000261405:c.2365A>G	VWF, VWF	1063856	benigna
C	No	1	Homozyg	1593	1595	99,87460	VWF	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000261405:p.His484Arg	ENST00000261405:c.1451A>G	VWF, VWF	1800378	benigna
T	No	1	Heterozyg	1363	2856	47,72408	VWF	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000261405:p.Val471Ile	ENST00000261405:c.1411G>A	VWF, VWF	1800377	benigna
C	No	1	Homozyg	899	900	99,88888	A2M	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000323929:p.Asn639Asp	ENST00000318602:c.1915A>G	A2M, A2M, A2M	226405	benigna
G	No	1	Heterozyg	788	1496	52,67379	CPB2, CPB2-AS1	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000181383:p.Ile347Thr	ENST00000181383:c.1040T>C	CPB2-AS1, CPB2-AS1, CPB	1926447	benigna
C	No	1	Homozyg	1367	1370	99,78102	SERPINA10	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000450896:p.Lys86Arg	ENST00000554723:c.257A>G	SERPINA10, SERPINA10, SI	941590	benigna
A	No	1	Homozyg	2194	2196	99,90892	SERPINA5	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000333203:p.Ser64Asn	ENST00000329597:c.191G>A	SERPINA5, SERPINA5, SERI	6115	benigna
T	No	1	Homozyg	974	975	99,89743	SERPINF2	ENSG00	ENST000(	ENST000(	ENSP000(	ENSP00000321853:p.Ala2Val	ENST00000324015:c.5C>T	SERPINF2, SERPINF2, SERF	2070862	benigna

GENOTYPE ←→ COAGULATION LAB PHENOTYPE ←→ CLINICAL PHENOTYPE

✓ To keep in mind

- ✓ Patients with inherited thrombophilia carry **increased RR of (first) VTE**. The real risk of VTE recurrence is mostly unknown.
- ✓ **Selecting patients for testing** and **knowing how to use the results** are essential in order to provide the best possible care for patients with VTE.
- ✓ **Unknown/new thrombophilic conditions need to be identified** and the impact on patients management evaluated in future studies
- ✓ Thromboprophylaxis strategies can **prevent** the majority of (provoked) VTE in patients with inherited thrombophilia (if known!) → **need for screening high risk patients**
- ✓ VTE **prevention and treatment strategies should be reviewed** on the basis of the knowledge of new inherited thrombophilia and new anticoagulants (DOACs) → **Studies needed**
- ✓ **CLINICAL THROMBOPHILIA & COAG PHENOTYPES & GENOTYPES:**

  A MATTER OF FURTHER INVESTIGATION

Padua –Scrovegni's Chapel (painted by Giotto 1304-1306 p.c.)