

Laboratory Tests of Hemostasis (Part 2)

Tests Of Thrombotic Disease and Hypercoagulability

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 - Amgen
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 - Novartis
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 - Alpine Immune Sciences

Learning Objectives:

Part 2: Laboratory Tests of Thrombotic Disease

- Describe the pathophysiology of heparin-induced thrombocytopenia and discuss the appropriate use of screening and confirmatory tests.
- Discuss the test principle and interpretation of confirmatory tests of the Lupus Anticoagulant assays.
- List appropriate laboratory evaluation of patients with suspected inherited thrombophilia.
- Discuss the effect of therapeutic anticoagulants on the reliability of results of clot based, chromogenic, immunologic and molecular tests.

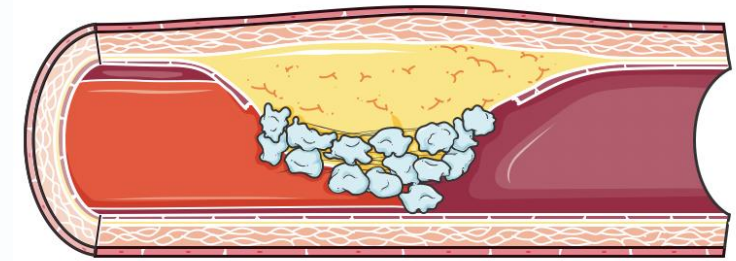
Material To Cover

1. *The Hemostatic Balance*
2. *Overview of The Coagulation Cascade and Testing*
3. *Functional and Immuno-assays*
4. *The Prothrombin and Activated Partial Thromboplastin Times*
5. *Other Tests:*
 - *Specific Factor Assays*
 - *Anti-Xa Heparin Assay*
 - *Thrombin Time*
 - *Fibrinogen Assay*
 - *D-Dimer*
 - *Thromboelastography (TEG) and Thromboelastometry (ROTEM)*
6. *Interpretation of Prolonged PT and/or aPTT Results*
7. *Tests Of Thrombotic Disease*
8. *Heparin Induced Thrombocytopenia/Thrombosis (HITT): Pathophysiology*
9. *Antiphospholipid Antibody Syndrome*
10. *Laboratory Testing for Thrombophilia (Hypercoagulable State)*
11. *APC-Resistance—Screening Assay For Factor V Leiden*
12. *Conditions That Impact Tests for Thrombotic Risk Factors.*
13. *If/When to Do Hypercoagulable Work-up*

Arterial vs Venous Thrombotic Disease

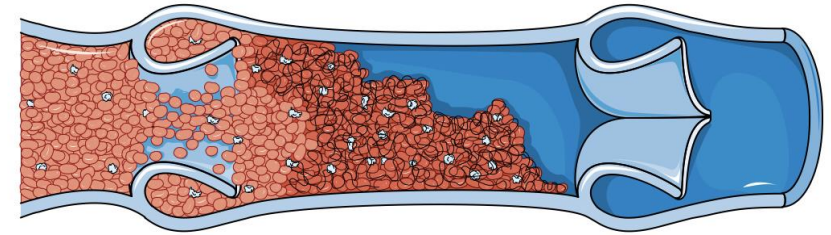
➤ Arterial thrombosis

- **Limited laboratory tests available**
- **Vascular Damage**
- **Atherosclerotic risk factors**
- **Platelet activity**



➤ Venous Thrombosis

- **Laboratory testing available for specific components, but no screening or global tests.**
- **Decreased regulation of coagulation**
- **Increased procoagulant activity**
- **Decreased fibrinolytic activity**
- **Non-Hematologic parameters**



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The Hemostatic Balance: Testing

Tests of Hemorrhagic Tendency

- PT
- APTT
- Fibrinogen
- Thrombin Time
- D-dimer
- Factor Assays
- VWD testing

Tests of Thrombotic Tendency (Routine/Standard)

- HIT
- Lupus Anticoagulant/Antiphospholipid Antibody
- Protein C
- Protein S
- Antithrombin
- FV Leiden
- PT G20210A
- Homocysteine?



Heparin Induced Thrombocytopenia/Thrombosis (HITT)

- *Full lecture to follow later in the year.*
- *Here we will focus on testing.*

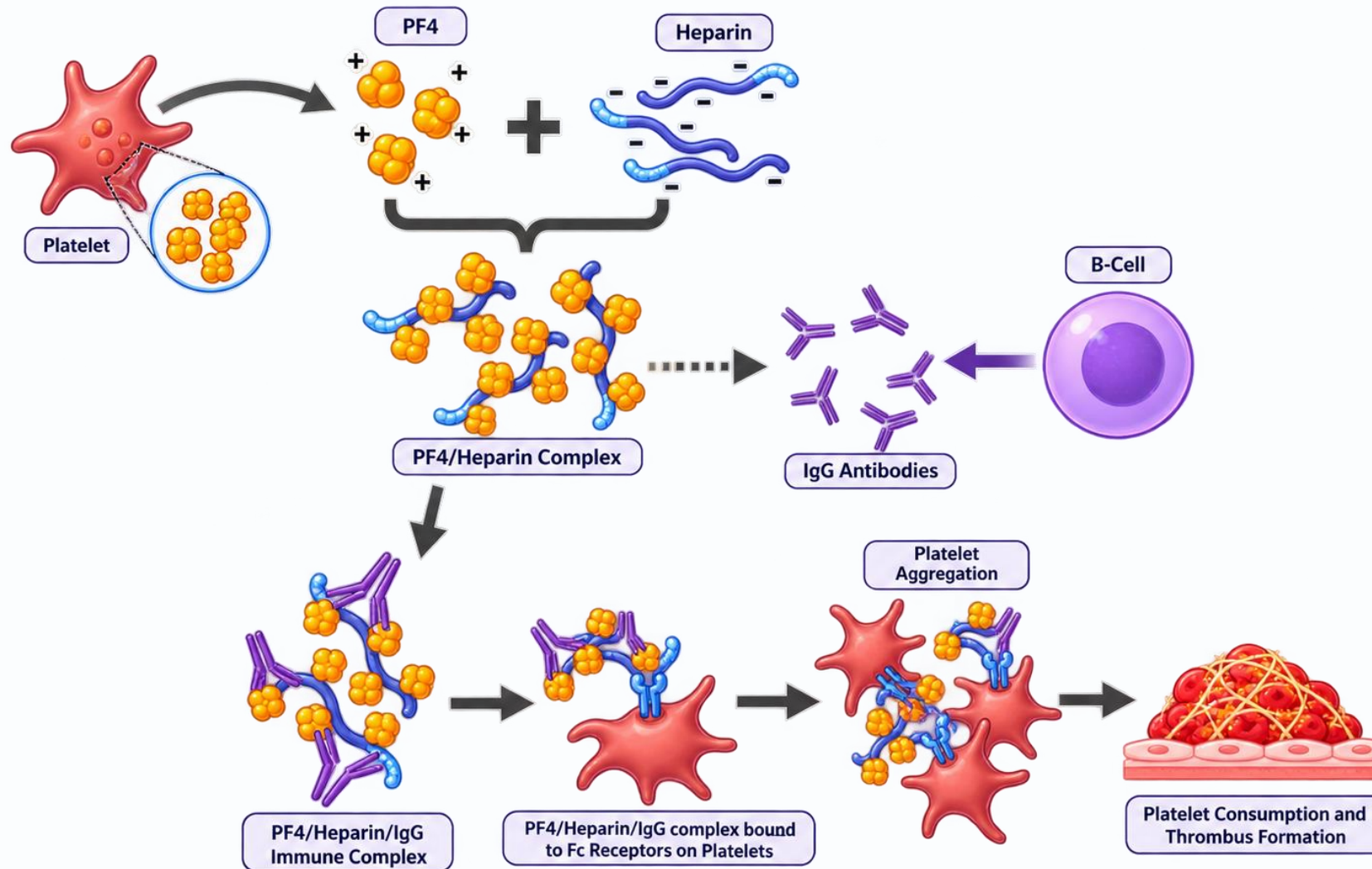
AVOID THIS PLEASE!



Cormack GM & Kaufman LJ. Journal of Medical Case Reports 2007, 1:13.

Noto, Tatsunori et al. Journal of Cardiology Cases, Volume 27, Issue 2, 56 - 59

Heparin-Induced Thrombocytopenia (HIT)



4T Scoring System for Pretest Probability

Points	2	1	0
Thrombocytopenia	>50% fall in PLT or PLT nadir of 20K-100K	30-50% fall in PLT or PLT nadir 10K-19K	<30% fall in PLT or PLT nadir of <10K
Timing	5-10 d post heparin [<1 day if previous heparin within 100 days]	Unclear or PLT fall after 10 days	PLT fall <5 days and without recent heparin
Thrombosis	New thrombosis, skin necrosis	Progressive or recurrent thrombosis, some skin lesions e.g., erythema	None
Other causes of Thrombocytopenia	None	Possible	Other causes clearly identified

Score ≤ 3 : < 5% chance of HIT
Score 4-5: Intermediate risk
Score ≥ 6 : Very high risk of HIT

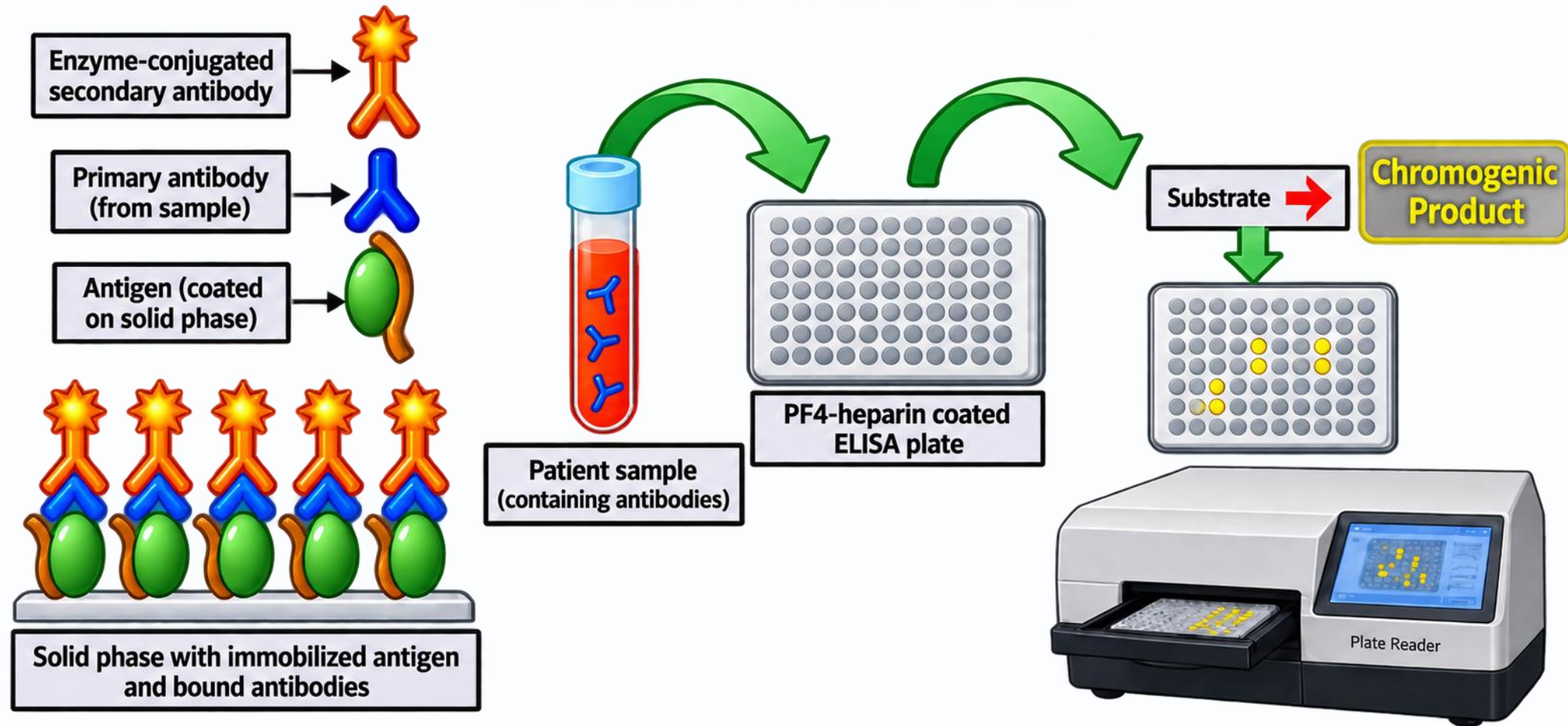
Cuker, A. et al. Blood 2012, 120(20): 4160–4167.

HIT Testing: Screening ELISA

- Antibodies to heparin-PF4 complexes
 - Polyspecific Assay: Combined IgG, IgA, IgM titers.
 - Monospecific Assay, IgG only titer (OD). More specific.
 - McFarland et al, Am J Hematol. 2012 Aug; 87(8): 776–781.
- High Negative Predictive Value*.
 - If the result is below the pre-specified cutoff, (Typically, <0.4 OD units) can be confident that HIT is not present.
 - If result if >0.4 , this does not indicate that HIT is present, but rather it still needs to be considered.

**A negative predictive value (NPV) is the probability that if the test is negative, the subject does not have the disease.*

Anti-PF4/heparin Enzyme-Linked Immunosorbent Assay (ELISA)



HIT/T ELISA Results

- Negative ELISA screen– HIT unlikely
- Positive ELISA screen- consistent with HIT/T in the appropriate clinical setting.
 - Does not mean that HIT/T is confirmed!
- Serotonin Release Assay: Confirmatory test.

Interpretation of HIT Titers In View of Serotonin Assay Confirmatory Results

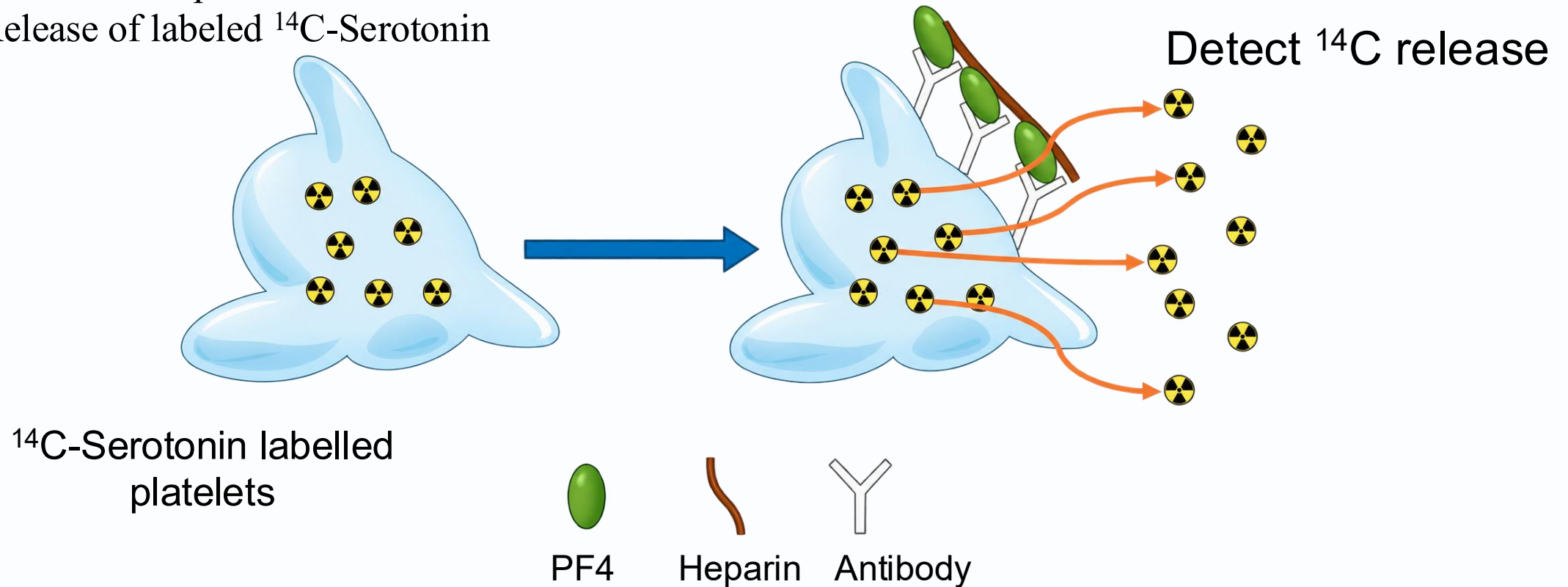
HIT Titer (OD)	Probability of Serotonin Assay POSITIVITY
< 0.4	~0 - <1%
0.4 - < 1.00	< 5%
1.00 - 1.50	~ 25%
1.50 - < 2.00	~ 50%
≥ 2.00	>90%

Warkentin et al. Thromb Haemost 2008;6:1304-12.

HIT/T Testing: Serotonin Release Assay

Uses fresh platelets, “loaded with ^{14}C -Serotonin” in dense granules.

1. Exposure to Antibody:Heparin:PF 4 Complex.
2. Activation of platelets
3. Release of labeled ^{14}C -Serotonin



Antiphospholipid Antibody Syndrome: Testing

- Full lecture to follow later in the year.
- Here we will focus on testing.

Lupus Anticoagulant

- Heterogeneous antibodies against phospholipid binding proteins, that “usually” prolong the aPTT.
- Prevalence of 1-4% in the general population. (Increases with Age)
- A key component of the Antiphospholipid Antibody Syndrome.
 - The LA carries the strongest association with thrombosis among the three criteria antibodies.
- Prolongs Screening aPTT
 - Clinical aPTT reagents are variably sensitive to LA
 - Normal aPTT does not rule out a LA
- Not usually associated with bleeding
- **Associated with arterial/venous/small vessel thrombosis**
- **Pregnancy: Recurrent fetal loss**
- Lupus anticoagulant–hypoprothrombinemia syndrome (LAHPS)
 - Patients may also have antibodies against prothrombin, but this is less common than antibodies to β 2-glycoprotein I.

References for Lupus Anticoagulant Slide

- Bertolaccini, M. L., Sciascia, S., Murru, V., Garcia-Fernandez, C., Sanna, G., & Khamashta, M. A. (2013). Prevalence of antibodies to prothrombin in solid phase (aPT) and to phosphatidylserine-prothrombin complex (aPS/PT) in patients with and without lupus anticoagulant. *Thromb Haemost*, 109(2), 207-213. doi:10.1160/th12-07-0527
- Guerin, J., Smith, O., White, B., Sweetman, G., Feighery, C., & Jackson, J. (1998). Antibodies to prothrombin in antiphospholipid syndrome and inflammatory disorders. *Br J Haematol*, 102(4), 896-902. doi:10.1046/j.1365-2141.1998.00876.x
- Pengo, V., Denas, G., Bison, E., Banzato, A., Jose, S. P., Gresele, P., . . . Tripodi, A. (2010). Prevalence and significance of anti-prothrombin (aPT) antibodies in patients with Lupus Anticoagulant (LA). *Thromb Res*, 126(2), 150-153. doi:10.1016/j.thromres.2010.05.018
- Pilania, R. K., Suri, D., Jindal, A. K., Kumar, N., Sharma, A., Sharma, P., . . . Singh, S. (2018). Lupus anticoagulant hypoprothrombinemia syndrome associated with systemic lupus erythematosus in children: report of two cases and systematic review of the literature. *Rheumatology International*, 38(10), 1933-1940. doi:10.1007/s00296-018-4127-9

Results in Patient with Lupus Anticoagulant: Immediate Acting inhibitor

Mixing Studies

aPTT	Patient	Normal Plasma (22.5-36.5")	50/50 mix
Immediate	62.6"	29.4"	60.7"
1 Hour Incubation @ 37°C	64.1"	29.3"	62.5"

Lupus Anticoagulant Insensitive aPTT Reagents

- aPTT-FS = “Factor Sensitive”
 - May have different names, depending on manufacturer.
 - "LA-insensitive" aPTT reagents are those whose phospholipid content is high enough to overcome the antibody, so they fail to prolong in the presence of lupus anticoagulant. The determinant of sensitivity is chiefly the phospholipid composition and concentration of the reagent,
 - Used to avoid inhibitory effect of LA on clot-based factor assays.
 - Used to rule out significant coagulation factor deficiencies in setting of LA.
 - Normal aPTT-FS result “rules out” a significant factor deficiency.
-
- aPTT: 62”
 - aPTT- FS: 32.1” (normal)

ISTH Guidelines for Lupus Anticoagulant Testing

(Devreese, KMJ et al. Journal of Thrombosis and Haemostasis, Volume 23, Issue 2, 2025, 731 - 744)

- **Two tests based on *different principles***
 - dRVVT (activates common pathway)
 - *sensitive* aPTT (low phospholipid and **silica** as activator)
 - A single test will detect only 60 -80% of cases
 - Both tests used together have a 20% false negative rate for low and intermediate titer lupus anticoagulants
- ***LA considered positive if one of the two tests gives a positive result.***
- False positive rate: ~10%
 - (Dembitzer et al, Am J Clin Pathol 2010; 134:764-773)

Confirmatory Test

- A confirmatory test involves the addition of excess phospholipid to shorten or correct the prolonged coagulation test.
- The lupus anticoagulant is characterized by the correction of prolonged clotting time with added phospholipid and not with control plasma.

The International Society of Thrombosis and Hemostasis (ISTH) states that four criteria must be met to confirm the presence of Lupus Anticoagulant.

1. (Screening test) Prolonged result in one of two coagulation tests that are phospholipid dependent such as PTT-LA or DRVVT.
2. Failure to correct (persistent prolongation) after 1:1 mixing with pooled normal plasma,
3. (Confirmatory test) Lack of prolonged time when adding additional phospholipid.
4. Ruling out other coexisting coagulation factor inhibitor such as factor VIII.

Results are considered valid only off interfering anticoagulants (VKA, heparin, DOACs) and should be interpreted cautiously during an acute-phase response, since elevated factor VIII and CRP can distort results.

- Bertolaccini, M. L., et al. (2013). Prevalence of antibodies to prothrombin in solid phase (aPT) and to phosphatidylserine-prothrombin complex (aPS/PT) in patients with and without lupus anticoagulant. *Thromb Haemost*, 109(2), 207-213.
doi:10.1160/th12-07-0527
- Devreese, K. M. J. (2020). How to Interpret Antiphospholipid Laboratory Tests. *Curr Rheumatol Rep*, 22(8), 38.
doi:10.1007/s11926-020-00916-5

Lupus Anticoagulant Testing: Interference by Presence of Anticoagulants.

- DOACs (dabigatran, rivaroxaban, apixaban) even at trough levels produce false positive results in 20-40% of patients.
 - (Ratzinger F, et al. Thromb. & Haemost. 2016; 116:235-240)
- Warfarin may produce false positive DRVVT test results
 - Ortel T. Am J Hematol. 2012 May; 87(Suppl 1): S75–S81.
- Heparin may produce false positive aPTT based test results
 - Ortel T. Am J Hematol. 2012 May; 87(Suppl 1): S75–S81.

Lupus Anticoagulant Testing:

- Assays may be transiently abnormal in setting of acute thrombosis.
- For diagnosis of APS, tests need to be repeated and confirmed to be persistent after 12 weeks.
- In addition to Lupus Anticoagulant, one can test for Antiphospholipid Antibodies (aPL) by ELISA for:
 - Anti-cardiolipin antibodies (aCL)
 - Beta-2 glycoprotein 1 antibodies (aβ2GPI)
 - (To be discussed at later date, in lecture on APS.)

Tests for Other Thrombophilias

Physiologic Anticoagulants

Antithrombin (AT)*

- With heparin/heparan as a cofactor, AT inactivates the activated serine protease enzymes of the coagulation system.
 - *Previously referred to as Antithrombin 3.
 - There is no antithrombin 1 or 2, so it is now referred to as Antithrombin.

Protein C/Protein S

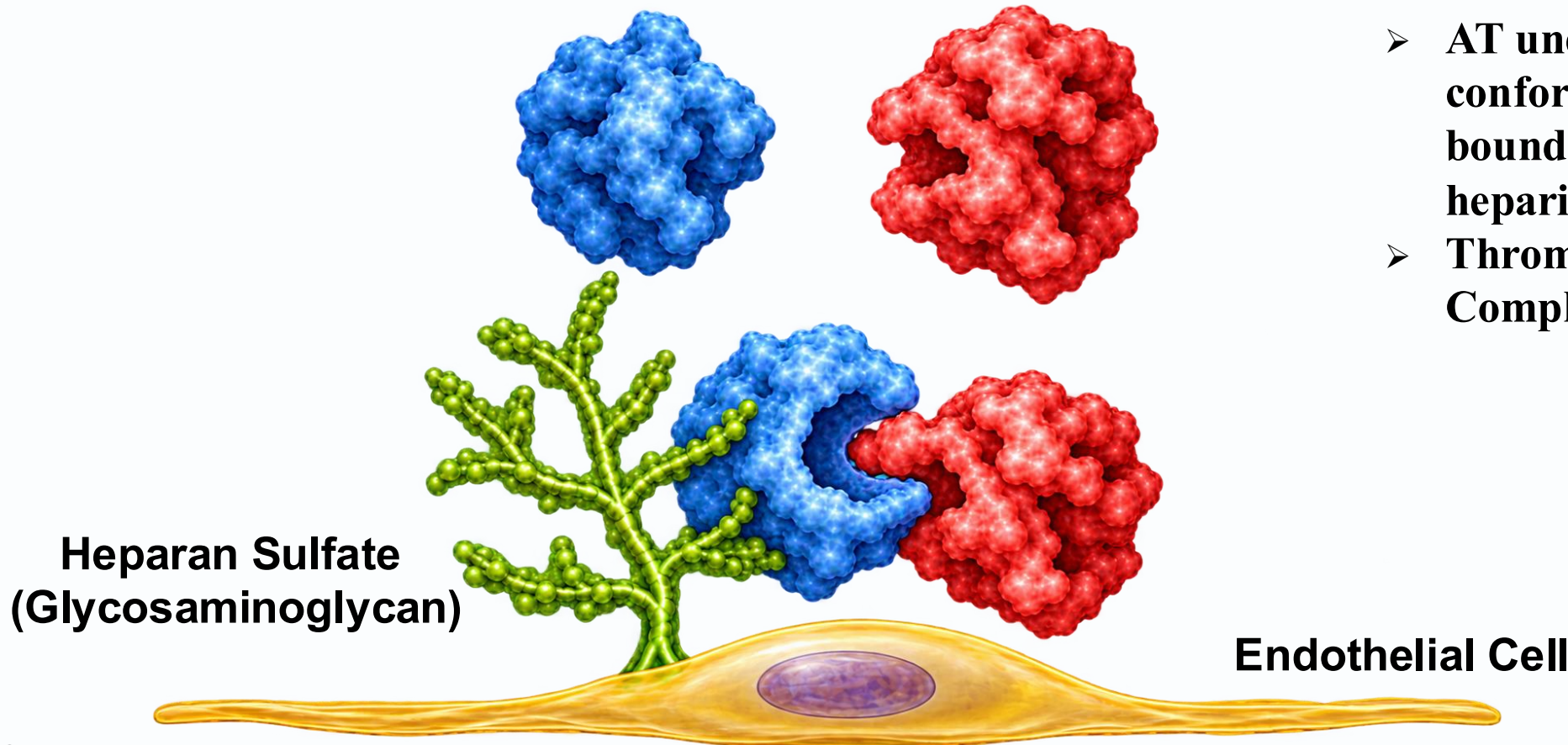
- Inactivates the activated cofactors of the coagulation system.
 - Factors Va, VIIIa
- Activate Protein C also has anti-inflammatory activity.

- **Can be measured functionally and antigenically.**
- **Deficiencies of AT, Protein C and Protein S are autosomal dominant. i.e. ~50% levels are associated with thrombotic tendency.**
- **Rare cases of homozygous Protein C deficiency: Purpura Fulminans**

Antithrombin: Glycosaminoglycan

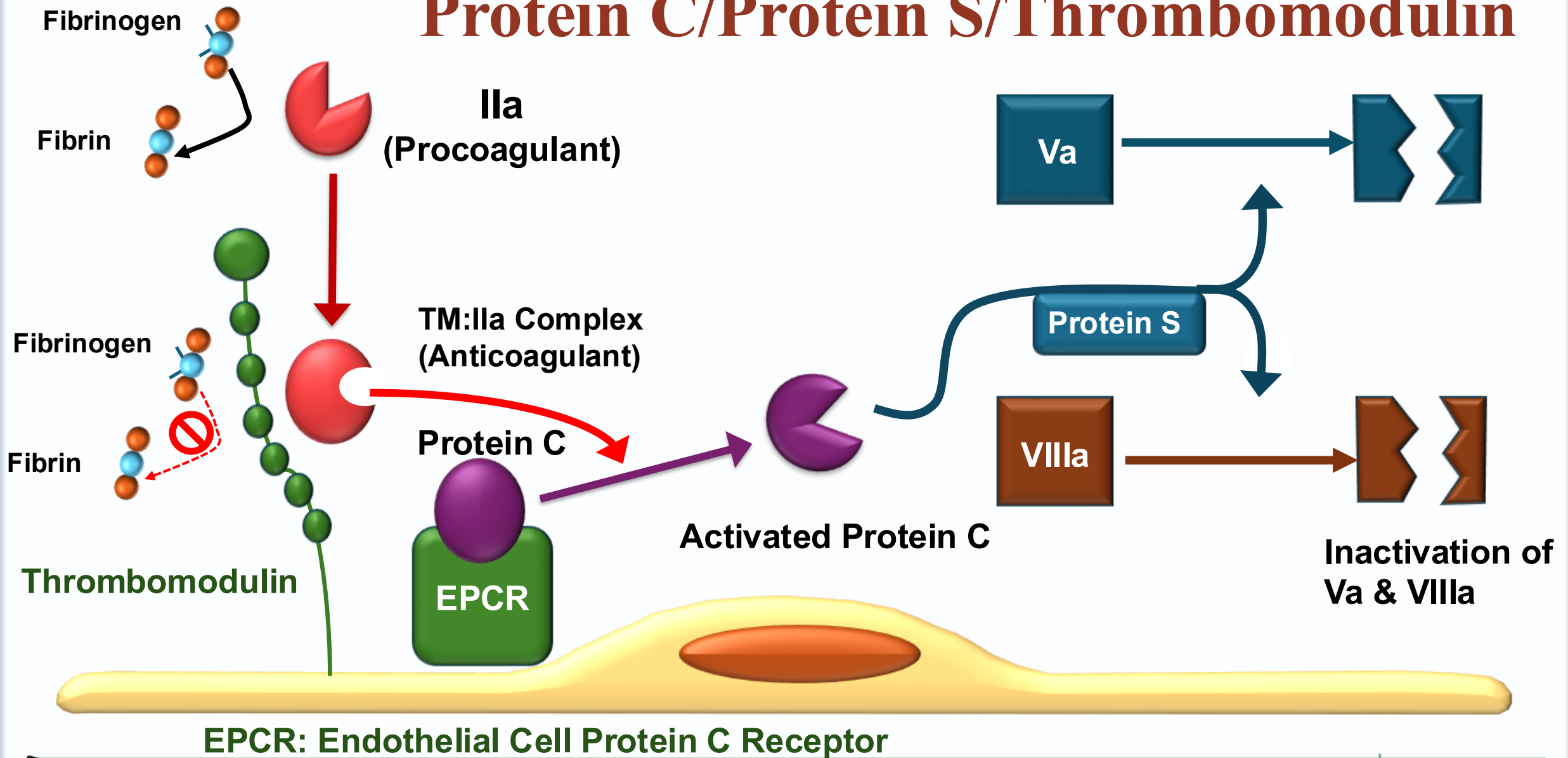
**Antithrombin:
Inactive Conformation**

Thrombin

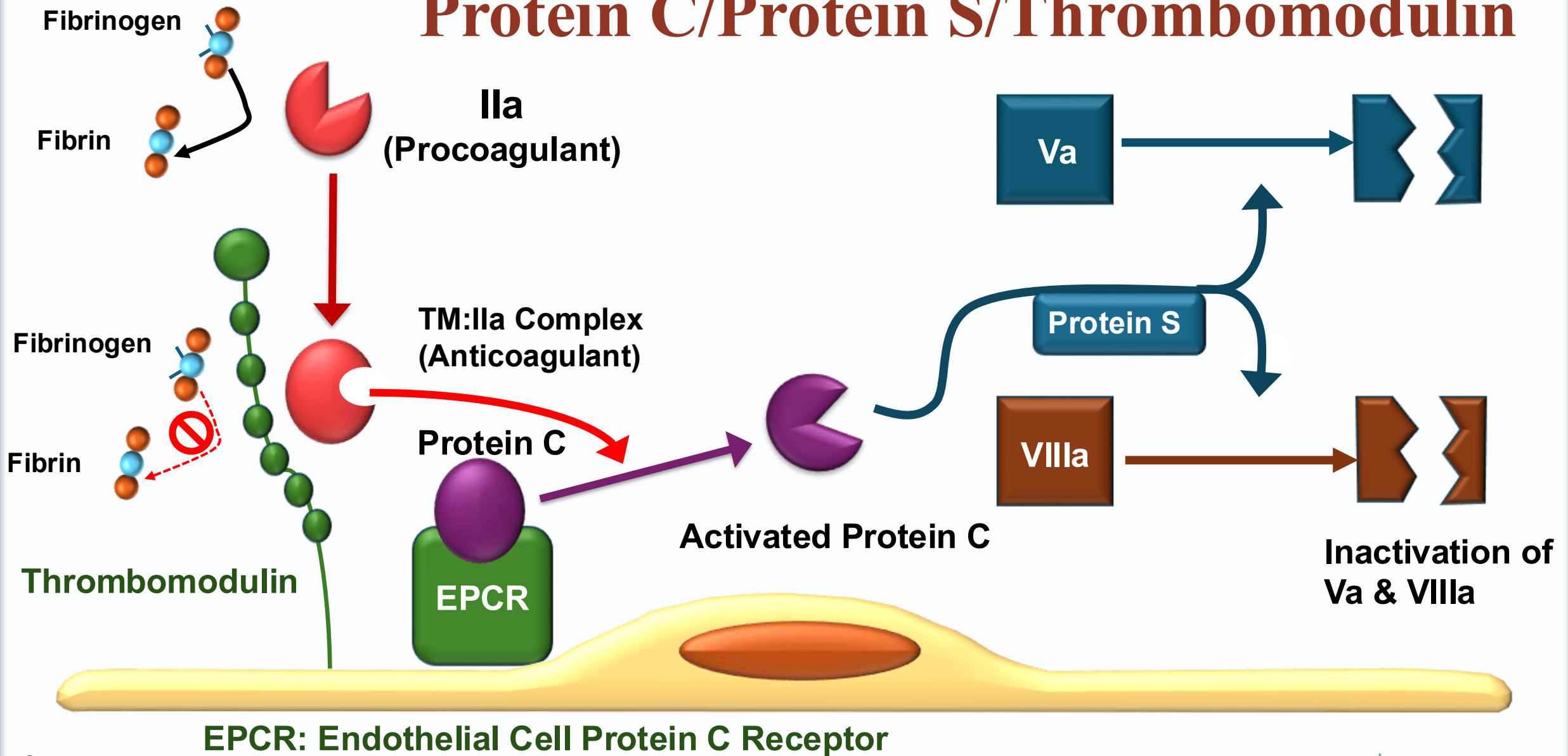


- AT undergoes a conformational change when bound to heparan or heparin.
- Thrombin:Antithrombin Complex Forms.

Protein C/Protein S/Thrombomodulin

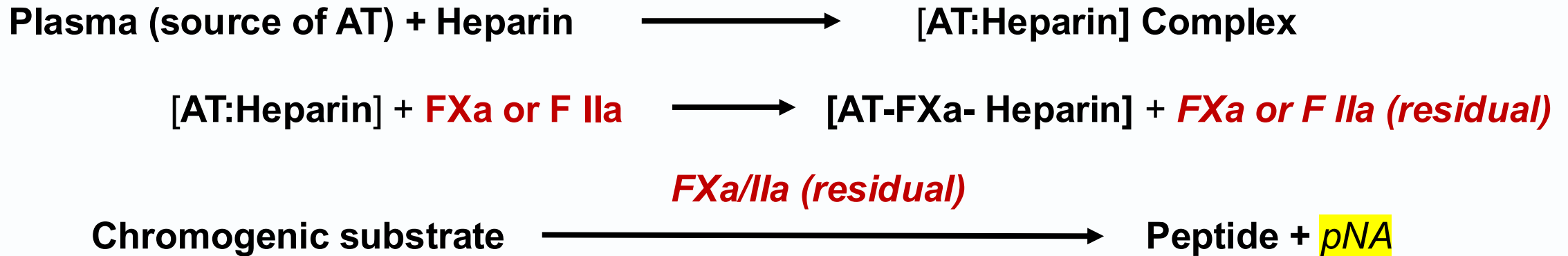


Protein C/Protein S/Thrombomodulin



Antithrombin Functional Assay

The assay measures functional AT levels in plasma.

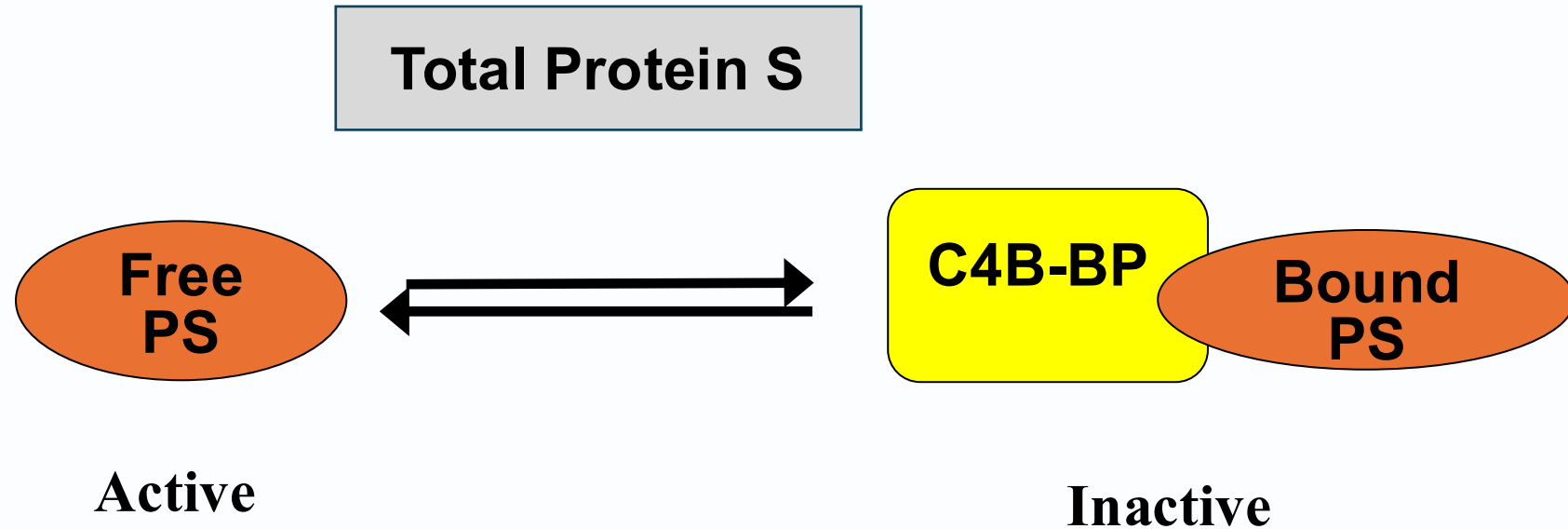


- Plasma (source of AT) is incubated with heparin and excess of Xa (or IIa).
- Residual Xa (or IIa) is determined by the rate of cleavage of the chromogenic substrate.
- The amount of product inversely proportional to the AT activity in the plasma sample.

Protein C Functional Assays

Clot-Based Assay	Chromogenic Assay
➤ Preatalytical variables ➤ <u>False low levels</u> ➤ FVIII (very high concentration) ➤ FV:Leiden ➤ Hyperlipidemia ➤ <u>False normal or high results</u> ➤ DOAC ➤ Heparin ➤ Lupus Anticoagulant	➤ Subject to fewer preanalytical variables. ➤ Detects most functional defects but not all.

Protein S Circulates in Two Forms



- Equilibrium between bound and free Protein S.
- Normally, ~60% of total Protein S is bound to C4b Binding Protein.
- Increase in C4B-BP reduces levels of free Protein S.

Three Types Protein S Assays

1. Clot-based functional PS assay—" activity" assay

Based on APC inactivation of FVa and FVIIIa.

2. Antigenic - Free PS assay (represents functional PS)

Free PS is adsorbed on the C4BP latex particle → triggers an agglutination reaction with the second latex reagent which is sensitized with a monoclonal antibody directed against human Protein S.

The degree of agglutination is directly proportional to the free PS concentration.

3. Antigenic - Total PS assay

Immunologic assay that measures PS bound to C4BBP + free PS.

Three Types of Protein S Deficiency

Type	PS (Activity)	PS (Free)	PS Total	C4B-BP
I	Decreased	Decreased	Decreased	Normal
II	Decreased	Normal	Normal	Normal
III	Decreased	Decreased	Normal	Elevated

- Type I protein S deficiency is a reduction in the level of free and total protein S.
- Type II deficiency is a reduction in the cofactor activity of protein S, with normal antigenic levels.
- Type III deficiency is a reduction in the level of free protein S, due to increase in C4B-BP.
 - (Acquired due to pregnancy, oral contraceptives).

Factor V:Leiden: Activated Protein C Resistance

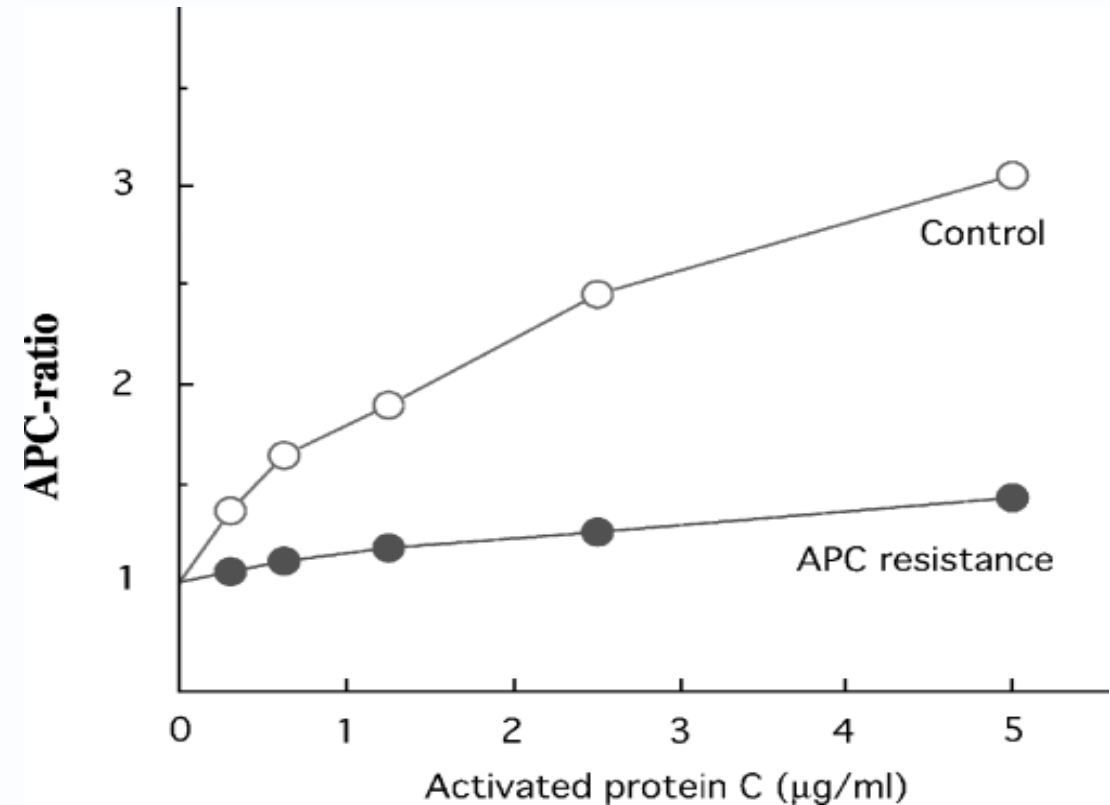
- Reduced anticoagulation response to aPC added to plasma in aPTT assay.
- Originally hypothesized a novel cofactor to aPC.
- Now understood as substrate (Factor Va) resistance to aPC.
- First described in 1993: Dahlbäck B, et al. *Proc Natl Acad Sci USA* 1993; **90**: 1004 - 8.

APC-Resistance—Screening Assay For Factor V Leiden

- Ratio of aPTTs (+/- APC)

$$\frac{(\text{aPTT with APC})}{(\text{aPTT without APC})}$$

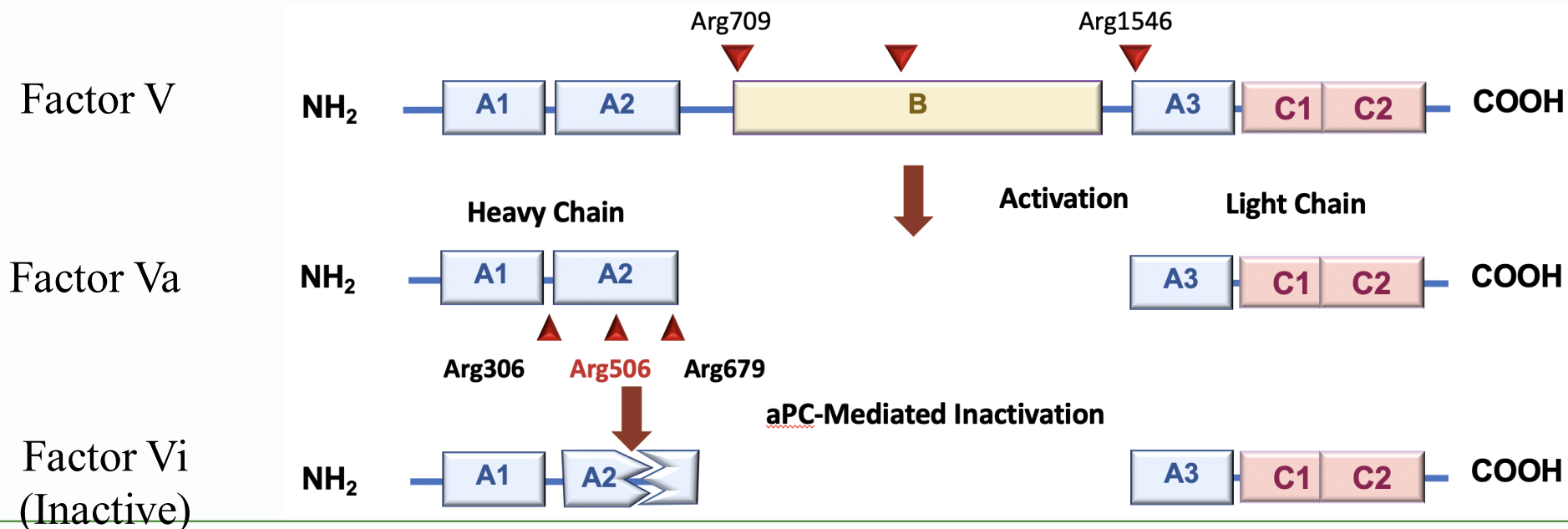
- Normal Ratio >2.0
- Assay is affected by
 - Lupus anticoagulant
 - DOAC
 - Cancer, pregnancy, inflammatory states
 - Elevated Factor VIII levels.
 - Other Factor deficiencies



Dahlbäck, B. J of Thrombosis Haemost, Volume: 1,
Issue: 1, Pages: 3-9, First published: 03 January 2003,
DOI: (10.1046/j.1538-7836.2003.00016.x)

Factor V:Leiden: Activated Protein C Resistance

- Inactivation of Factor Va involves four sequential cleavages of the membrane-bound procofactor at Arg306, Arg506, Arg679, and Lys994.
- Factor V:Leiden polymorphism (G1691A (R506Q)) is associated with activated protein C resistance, prolonged/enhanced activity of Factor Va, and thrombotic tendency.
- Now routinely tested by PCR/molecular tools.



Prothrombin Gene Mutation: (Prothrombin G20210A)

- G-to-A point “polymorphism” at position 20210 in the 3' untranslated region of the prothrombin (factor II) gene.
- The polymorphism does not affect the protein-coding region (exons) of the gene.
- Increases mRNA half-life and Prothrombin levels.
 - Poort et al Blood. 1996;88(10):3698.

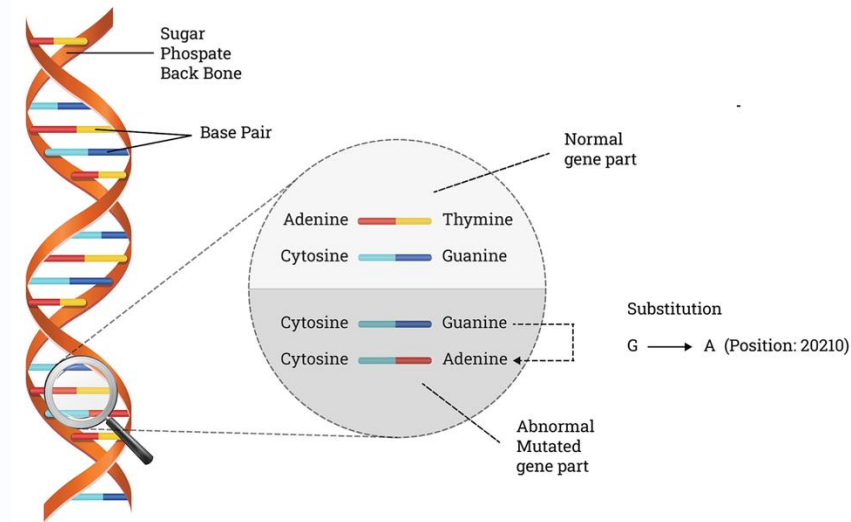


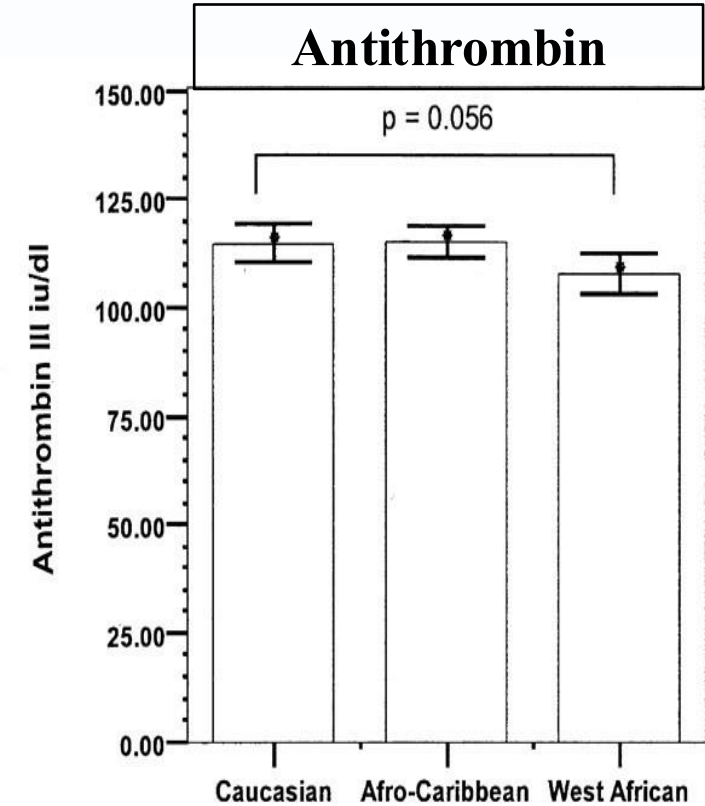
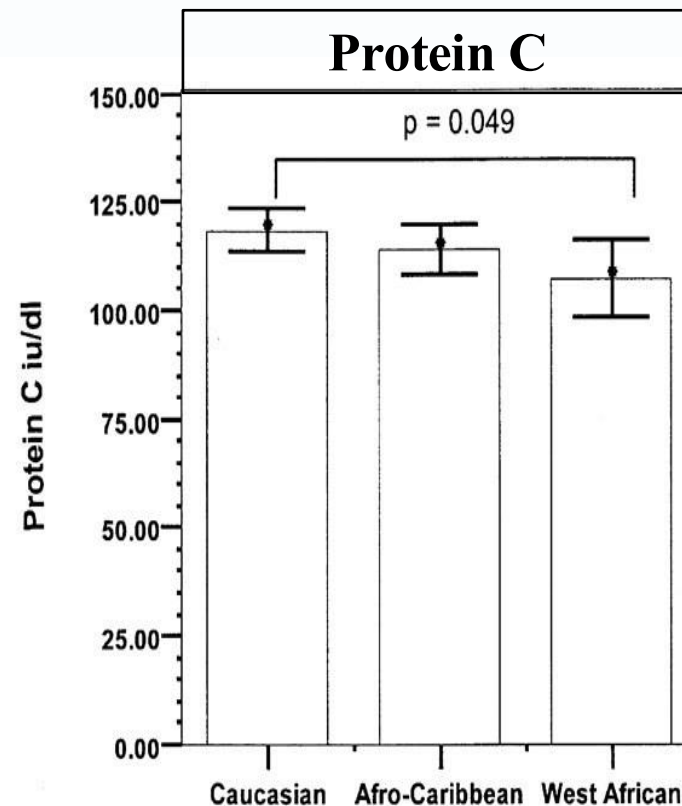
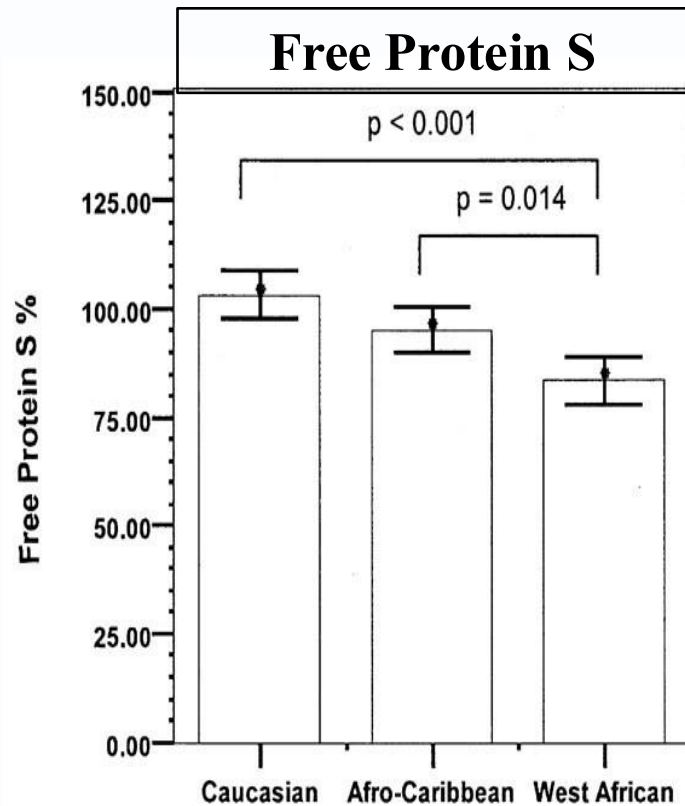
FIGURE 2: Prothrombin G20210A Mutation

G- Guanine & A- Adenine

Adapted from: www.coagulationconversation.com/medical/risk-factors-thrombophilia-prothrombin-20210-mutation

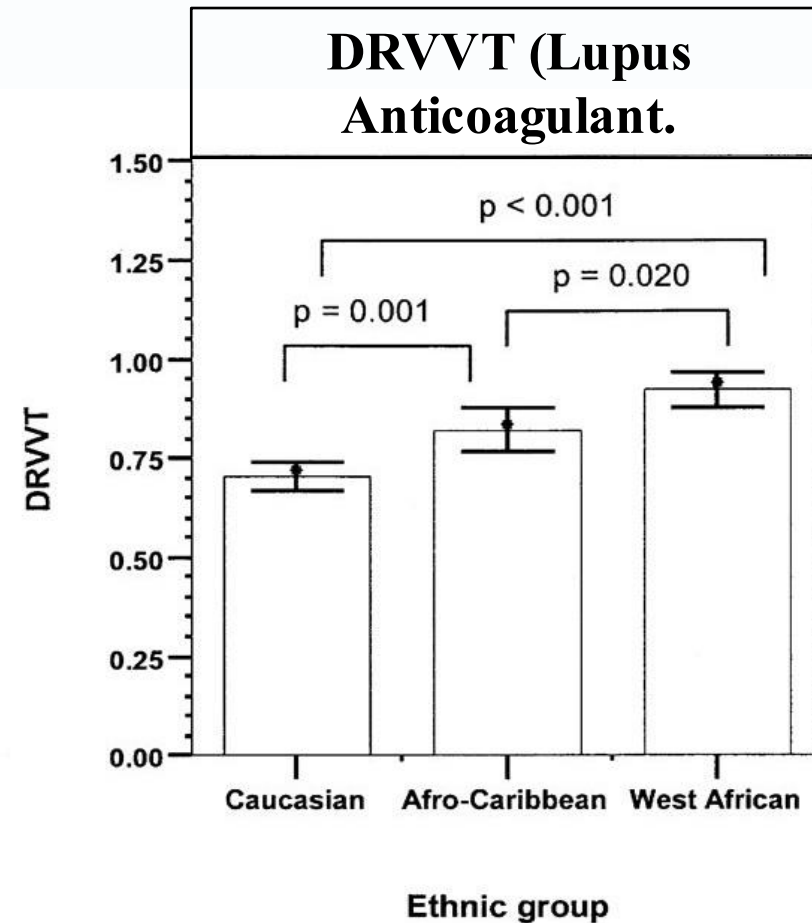
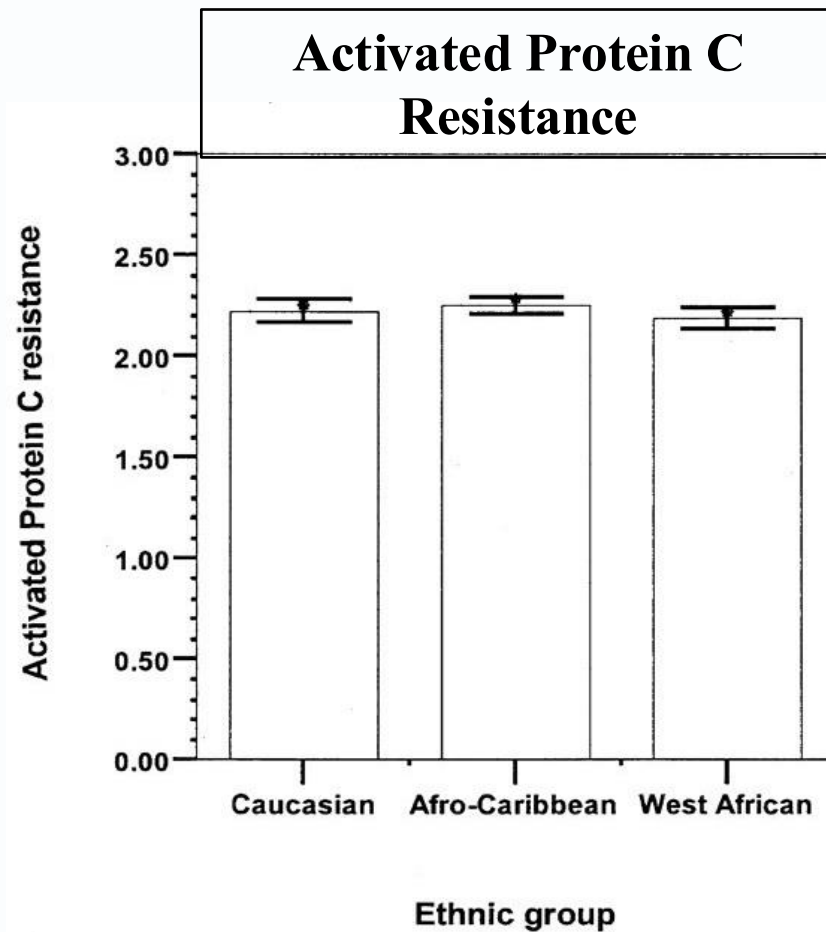
Poudel S, Zeb M, Kondapaneni V, et al. (December 08, 2020) Association of G20210A Prothrombin Gene Mutation and Cerebral Ischemic Stroke in Young Patients. Cureus 12(12): e11984. DOI 10.7759/cureus.11984

Ethnic Differences in Markers of Thrombophilia



Paula Jerrard-Dunne. Stroke. Ethnic Differences in Markers of Thrombophilia, Volume: 34, Issue: 8, Pages: 1821-1826, DOI: (10.1161/01.STR.0000083049.65008.5F)

Ethnic Differences in Markers of Thrombophilia



Paula Jerrard-Dunne. Stroke. Ethnic Differences in Markers of Thrombophilia, Volume: 34, Issue: 8, Pages: 1821-1826, DOI: (10.1161/01.STR.0000083049.65008.5F)

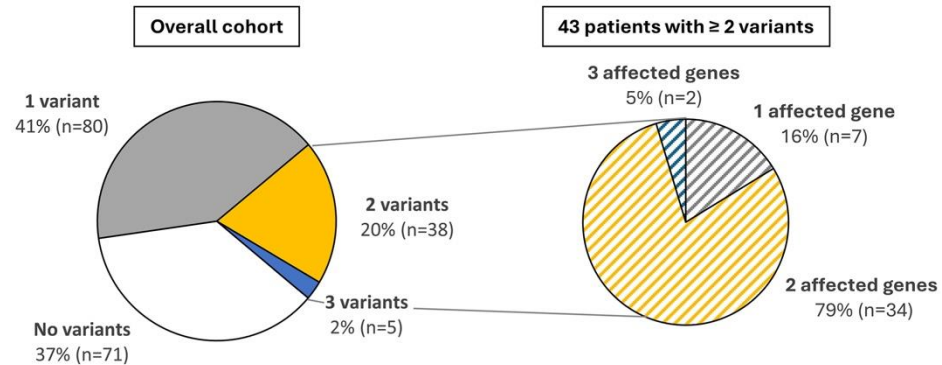
Laboratory Testing for Thrombophilia (Hypercoagulable State)

Laboratory Testing for Thrombophilia (Hypercoagulable State)

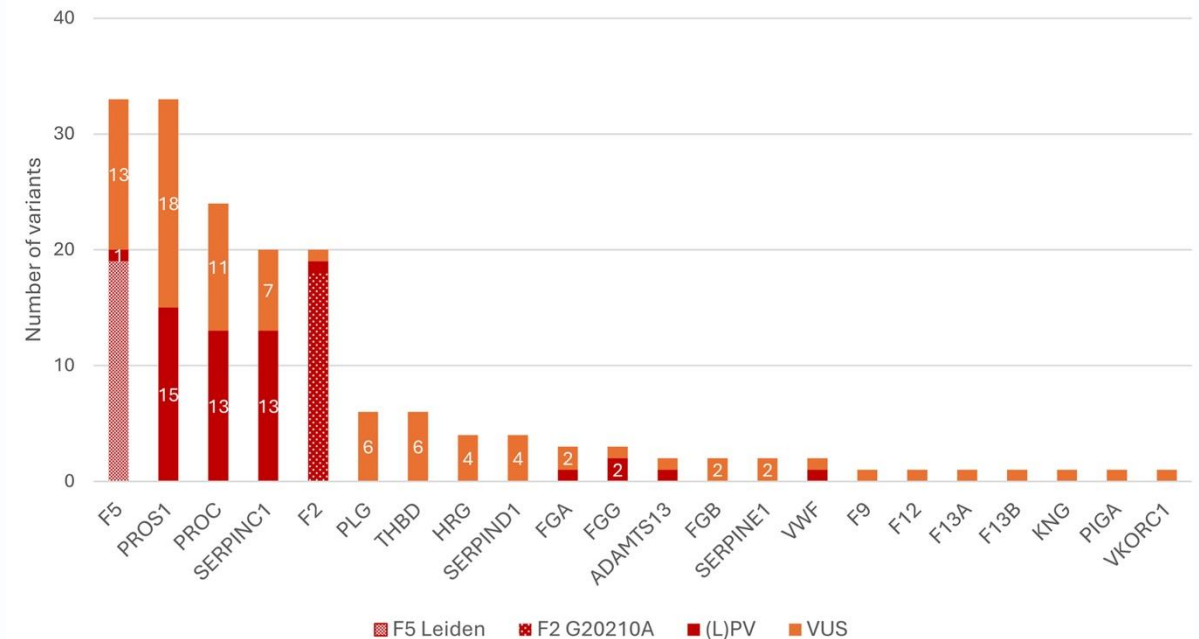
- No Screening test exists.
 - Requires a panel of tests.
 - Diagnosis of an abnormality can be made in ~50% of patients, depending on family history and presence/absence of provoking factors for the index venous thromboembolism.
1. Antiphospholipid Antibody Syndrome.
 - A. Lupus anticoagulant
 - B. Anti cardiolipin antibodies
 - C. Beta 2 glycoprotein I antibodies
 2. Antithrombin (AT)
 3. Protein C
 4. Protein S
 5. F V Leiden
 6. Prothrombin G20210A
 7. Homocysteine
(Controversial if should be tested)

Multigene Panel for Thrombophilia Testing in Venous Thromboembolism

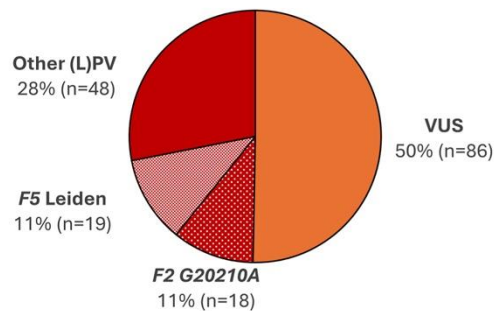
A Classification of Patients according to Number of Detected Variants and Affected Genes



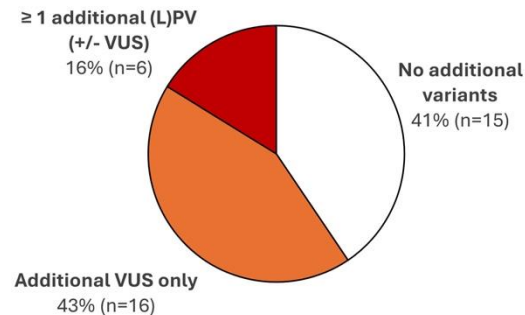
C Overview of Detected Variants



B Classification of 171 Detected Variants



D Gene results in F5 Leiden and/or F2 G20210A carriers



Verstraete, Andreas et al. Journal of Thrombosis and Haemostasis, 2025 Volume 23, Issue 6, 1838 - 1849

Multigene Panel for Thrombophilia Testing in VTE

- FV (Leiden)
- PROS1 (Protein S)
- PROC (Protein C)
- SERPINC1 (Antithrombin)
- F2 (Prothrombin)
- PLG (Plasminogen)
- THBD (Thrombomodulin)
- HRG (Heregulin)
- SERPIND1 (Heparin Cofactor 2)
- FGA (Fibrinogen alpha)
- FGG (Fibrinogen gamma)
- ADAMTS13
- FGB (Fibrinogen beta)
- SERPINE1 (plasminogen activator inhibitor-1 (PAI-1))
- VWF (von Willebrand Factor)
- F9 (Factor IX)
- F12 (Factor XII)
- F13A (Factor XIII A Chain)
- F13B (Factor XIII B Chain)
- KNG (High Molecular Weight Kininogen)
- PIGA (phosphatidylinositol glycan anchor biosynthesis class A)
- VKORC1 (vitamin K epoxide reductase complex subunit 1)

Verstraete, Andreas et al. Journal of Thrombosis and Haemostasis, 2025 Volume 23, Issue 6, 1838 - 1849

When to Evaluate for Thrombophilia?

- Levels of physiologic anticoagulants may be impacted by anticoagulants, acute venous thromboembolism, inflammation, acute illnesses.
 - Results of thrombophilia testing will not impact initial choice of anticoagulant.*
 - THEREFORE, testing in setting of an acute thrombosis is not typically indicated.
-
- ❖ *In Antiphospholipid Antibody Syndrome, with arterial or atypical site venous thrombosis, DOAC is not appropriate.*
 - ❖ *If patient has elevated baseline aPTT and/or thrombosis in atypical site, it may be appropriate to test for Antiphospholipid Antibody Syndrome at initial presentation.*

Why Do Hypercoagulable Work-up?

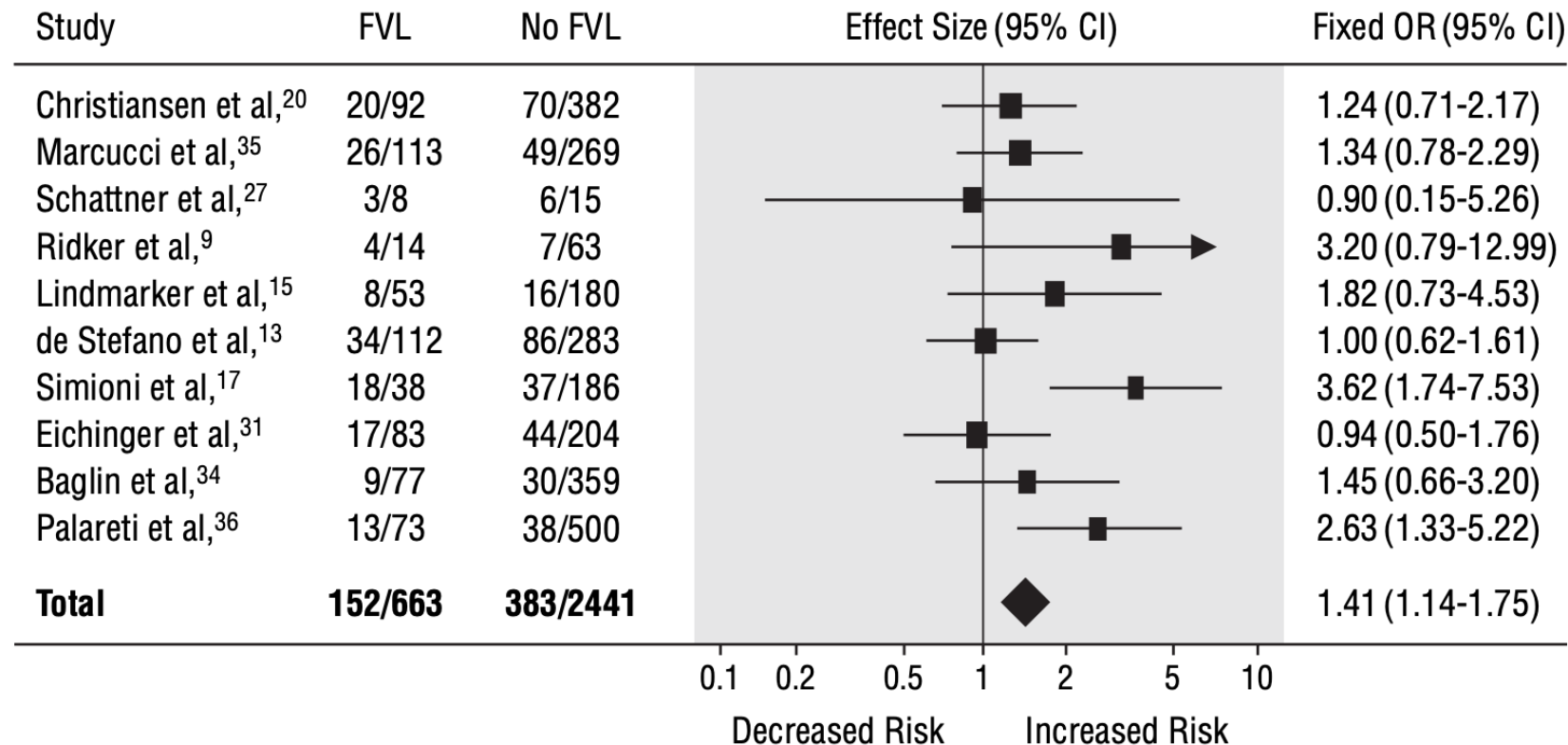
- **If it will impact choice of which anticoagulant should be used, or Duration of anticoagulation.**
- In Antiphospholipid Antibody Syndrome, with arterial events, DOACs are inferior to warfarin.
 - Pastori D, et al. Front Cardiovasc Med. 2021 Aug 3;8:715878.
- What about the other thrombophilias?
 - Evidence is evolving.

Impact of Thrombophilia on the Risk of a VTE

- Impact is mostly on initial VTE rates.
- Estimates are that hereditary thrombophilia confers only a modest ~1.5-fold increase in the risk of recurrent VTE, and this magnitude is small enough that ASH generally does not recommend testing to guide anticoagulation duration.
- “The risk for recurrent VTE in patients with thrombophilia vs patients without thrombophilia:
 - Any hereditary thrombophilia (RR, 1.56; 95% CI, 1.31-1.86)
 - APLAs/lupus anticoagulants (RR, 1.92; 95% CI, 0.99-3.72)
- ASH does carve out **higher-risk scenarios where the result may matter:**
 - VTE provoked by a nonsurgical major transient risk factor
 - Cerebral venous thrombosis when the default is to stop at 3–6 months
 - Testing being suggested only when a positive result would justify indefinite anticoagulation.
- Middeldorp S, et al. American Society of Hematology 2023 guidelines for management of venous thromboembolism: thrombophilia testing. Blood Adv. 2023 Nov 28;7(22):7101-7138. doi: 10.1182/bloodadvances.2023010177. PMID: 37195076; PMCID: PMC10709681.

Risk of Recurrent Venous Thromboembolism in Patients With Common Thrombophilia: A Systematic Review

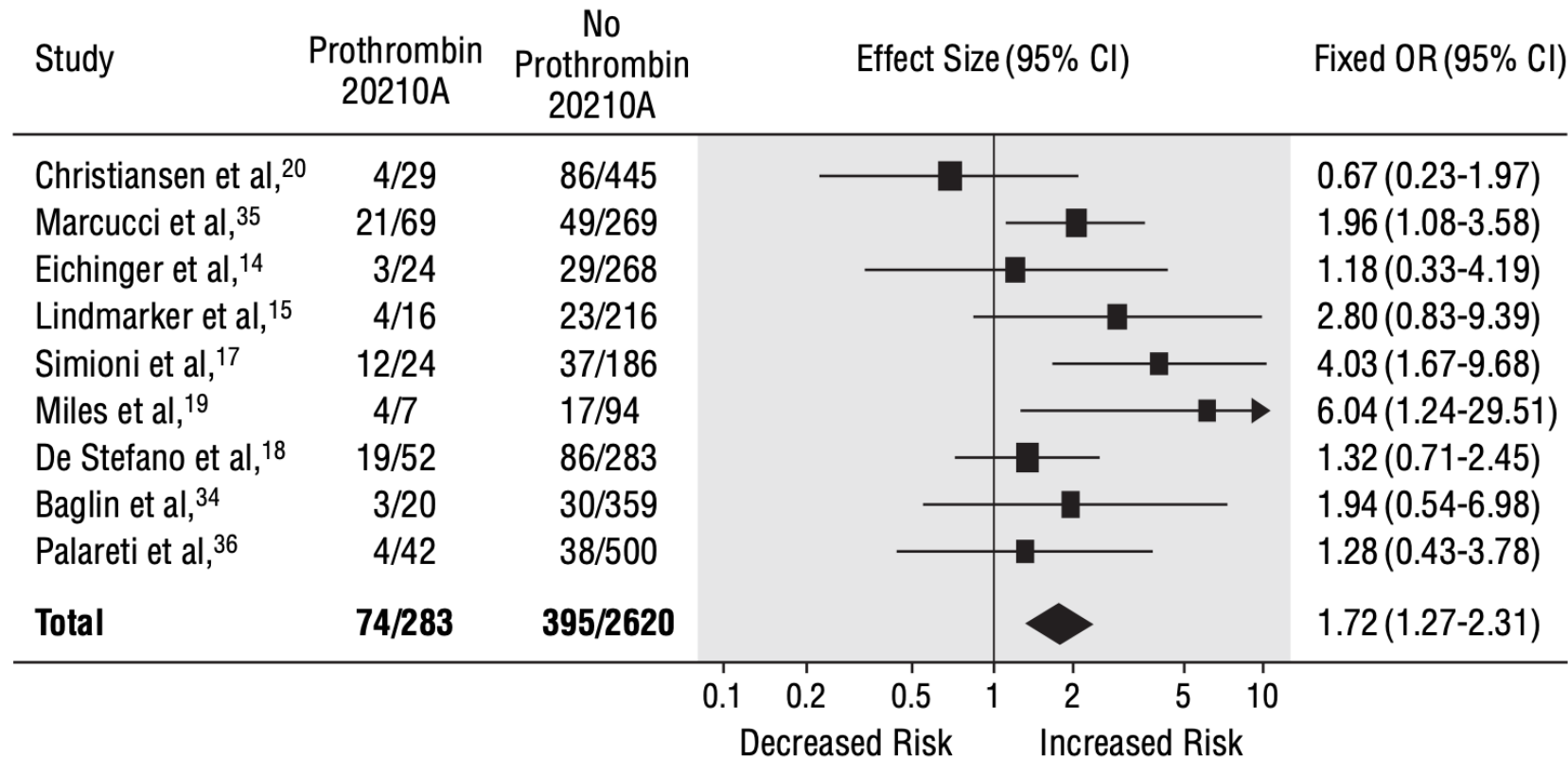
Heterozygous Factor V:Leiden



➤ Ho WH. Et al Arch Intern Med. 2006;166:729-736

Risk of Recurrent Venous Thromboembolism in Patients With Common Thrombophilia: A Systematic Review

Heterozygous Prothrombin G20210A



➤ Ho WH. Et al Arch Intern Med. 2006;166:729-736

Impact of Thrombophilia on the Risk of a Recurrent VTE

Thrombophilia	Prevalence, median % (min-max)	RR for VTE recurrence, positive vs negative (95% CI)
Any Thrombophilia	38.0 (21.6-59.5)	1.65 (1.28-2.47)
FVL homozygous	1.5 (0.3-3.1)	2.10 (1.09-4.06)
FVL heterozygous	17.5 (4.1-34.8)	1.36 (1.19-1.57)
PGM	6.1 (1.4-16.3)	1.34 (1.05-1.71)
Antithrombin (AT) deficiency	2.2 (0.2-8.7)	2.07 (1.50-2.87)
Protein C (PC) deficiency	2.5 (0.7-8.6)	2.13 (1.26-3.59)
Protein S (PS) deficiency	2.3 (0.7-7.3)	1.30 (0.87-1.94)
AT, PC, or PS deficiency	7.0 (2.5-18.4)	1.62 (1.17-2.23)
APLA	9.7 (1.9-19.4)	1.92 (0.99-3.72)

Middeldorp S, et al. American Society of Hematology 2023 guidelines for management of venous thromboembolism: thrombophilia testing. Blood Adv. 2023 Nov 28;7(22):7101-7138. doi: 10.1182/bloodadvances.2023010177. PMID: 37195076; PMCID: PMC10709681.

Thrombophilia and Risk of Recurrent VTE

Thrombophilia	Effect on recurrent VTE	References
Heterozygous FVL	OR ~1.4–1.56; absolute ~7–14%/yr in some cohorts	[1-3]
Heterozygous PGM (prothrombin G20210A)	~1.4-fold; often non-significant in individual studies	[1, 4]
Homozygous FVL / compound heterozygous FVL+PGM	~2–3-fold	[3, 5]
Antithrombin deficiency	OR ~2–4; absolute ~8.8%/yr off anticoagulation	[6]
Protein C deficiency	OR ~2.9 for recurrence; ~5.1%/yr	[6]
Protein S deficiency	OR ~2.5 (CI often crosses 1.0); ~6.5%/yr	[6]
Antiphospholipid syndrome (acquired)	RR ~1.9–2+; high-risk profiles higher	[7]

Thrombophilia and Risk of Recurrent VTE (References)

1. Risk of Recurrent Venous Thromboembolism in Patients With Common Thrombophilia: A Systematic Review. Archives of Internal Medicine. 2006. Ho WK, Hankey GJ, Quinlan DJ, Eikelboom JW.SR
2. Factor V Leiden. The Journal of the American Medical Association. 2025. Martin KA, Cushman M.
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4. Thrombophilia Testing and Venous Thrombosis. The New England Journal of Medicine. 2017. Connors JM.
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7. American Society of Hematology 2023 Guidelines for Management of Venous Thromboembolism: Thrombophilia Testing. Blood Advances. 2023. Middeldorp S, Nieuwlaat R, Baumann Kreuziger L, et al.
8. The Risk of Recurrent Venous Thromboembolism in Heterozygous Carriers of Factor V Leiden and a First Spontaneous Venous Thromboembolism. Archives of Internal Medicine. 2002. Eichinger S, Weltermann A, Mannhalter C, et al.Observational
9. Outcome of Patients With Venous Thromboembolism and Factor v Leiden or Prothrombin 20210 Carrier Mutations During the Course of Anticoagulation. The American Journal of Medicine. 2017. Tzoran I, Papadakis M, Brenner B, et al.
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ASH Recommendations for Thrombophilia Testing for Patients with Symptomatic VTE

Test for Thrombophilia, & Indefinite Anticoagulant for Patients with Thrombophilia

- VTE provoked by nonsurgical major transient risk factor.
- VTE provoked by pregnancy or postpartum.
- VTE associated with use of Combined Oral Contraceptives

Do not test for thrombophilia

- Unprovoked VTE*
- VTE provoked by surgery**

* Unprovoked VTE: Indefinite anticoagulation recommended for most patients.

** VTE provoked by surgery: most patients with VTE provoked by temporary risk factors will discontinue anticoagulant therapy after completion of the primary treatment.

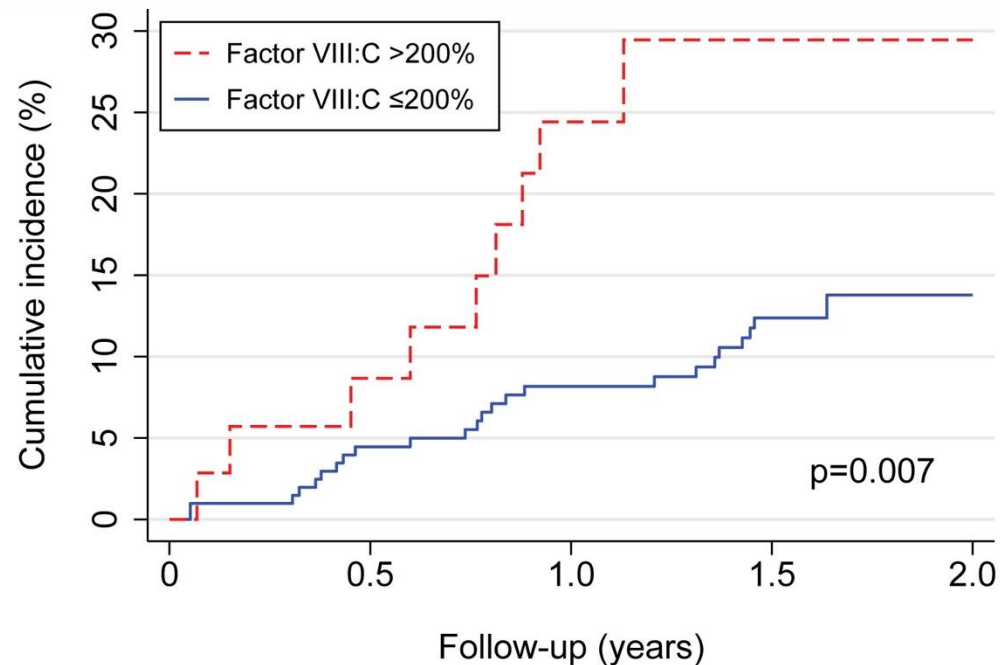
Middeldorp S, et al. American Society of Hematology 2023 guidelines for management of venous thromboembolism: thrombophilia testing. Blood Adv. 2023 Nov 28;7(22):7101-7138. doi: 10.1182/bloodadvances.2023010177. PMID: 37195076; PMCID: PMC10709681

Thrombophilia and Outcomes of Venous Thromboembolism in Older Patients.

➤ **Thrombophilic factor:**

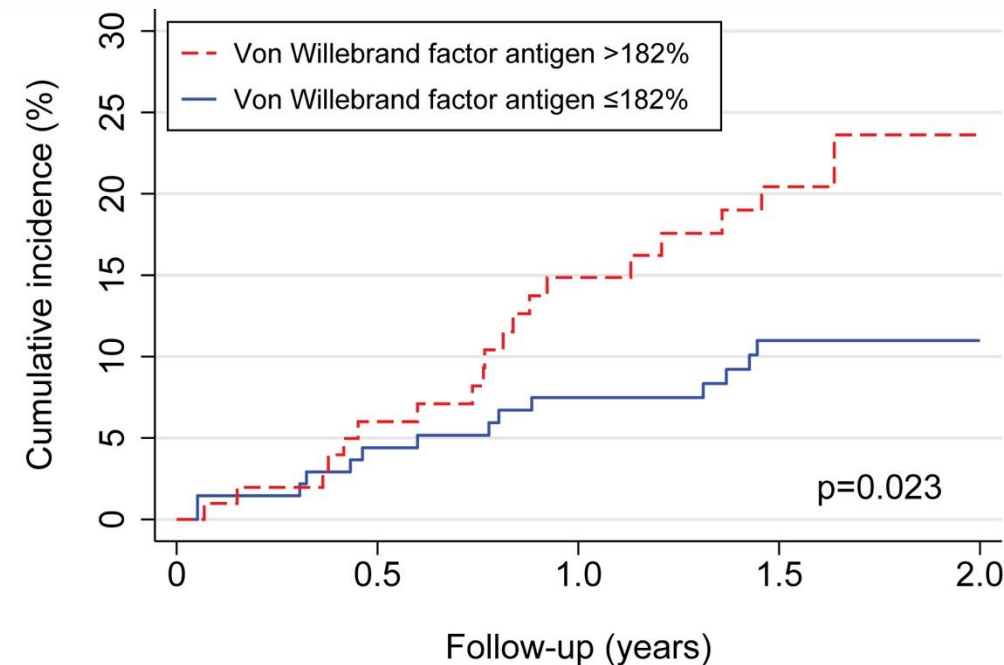
- Antithrombin deficiency
- Protein C deficiency
- Protein S deficiency
- Elevated von Willebrand factor Ag (>182%)
- Elevated coagulant activity of factor VIII (FVIII:C) (>200%)
- Elevated FIX:C
- Elevated FXI:C
- Elevated homocysteine
- Elevated fibrinogen
- Anti-phospholipid antibodies
- Factor V Leiden variant
- Prothrombin G20210A polymorphism

- 240 patients aged ≥ 65 years, 1 year after a blood clot.
- **Many parameters are not routinely assessed as thrombophilia.**
- Mean et al. Res Pract Thromb Haemost. 2023;7:e100015

A

Number at risk

FVIII:C >200%	36	30	19	5	1
FVIII:C ≤200%	204	185	158	129	85

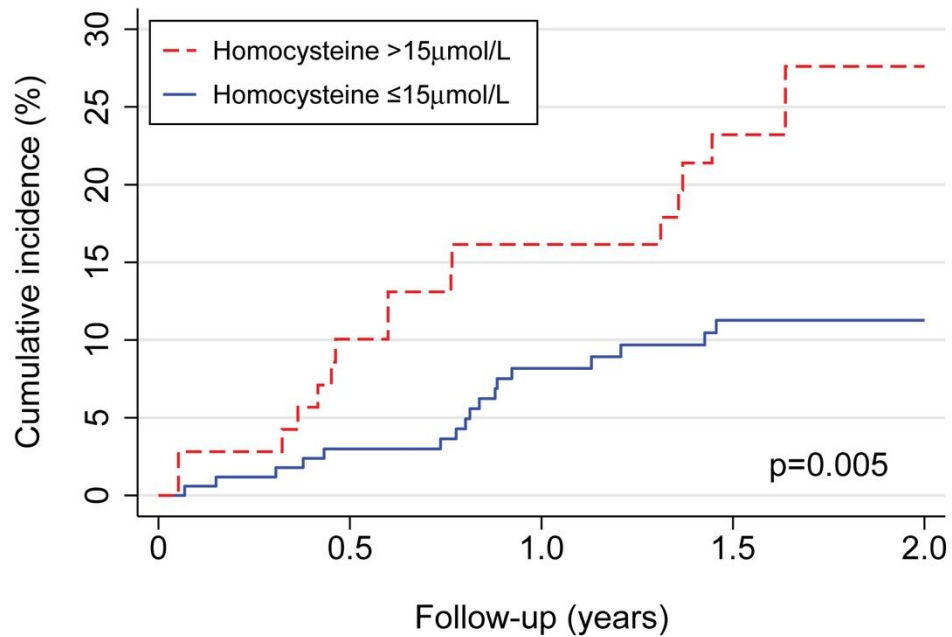
B

Number at risk

VWF:Ag >182%	103	89	68	51	34
VWF:Ag ≤182%	137	126	109	83	52

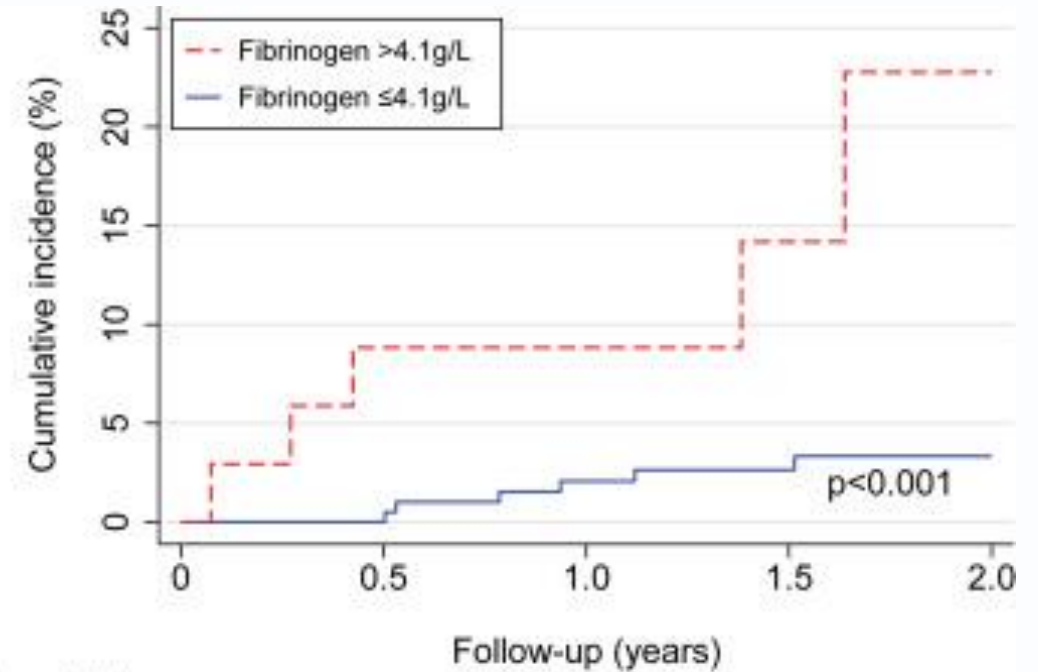
Mean et al. Res Pract Thromb Haemost. 2023;7:e100015

C



Number at risk

Hc >15μmol/L	71	60	50	35	21
Hc ≤15μmol/L	169	155	127	99	65



Number at risk

Fg >4.1g/L	34	27	20	11	4
Fg ≤4.1g/L	206	198	175	143	96

Mean et al. Res Pract Thromb Haemost. 2023;7:e100015

Comparison of Incidence Rates of VTE Recurrence Between Patients With 0, 1 or ≥ 2 Thrombophilia Risk Factors.

Number of thrombophilic risk factors	Number of patients	Number of events/ patient-y	IR (95% CI) per 100 patient-y	P value
0 thrombophilic risk factors	52	4/97	4.1 (1.5-11.0)	-
1 thrombophilic risk factor	75	10/130	7.7 (4.1-14.3)	.30
≥ 2 thrombophilic risk factors	113	25/178	14.1 (9.5-20.8)	.01

Mean et al. Res Pract Thromb Haemost. 2023;7:e100015

- Do we need to reconsider how to view thrombophilias?
- Which ones to test?
- Is the important consideration the number of abnormalities, rather than presence of one versus none?

Thrombophilia and Stroke

- A case–control study of 219 patients with a first ischemic stroke.
- The prevalence of any thrombophilia
 - Stroke Cases: 14.7% (95% CI, 9.9% to 19.5%) among cases
 - Control subjects: 11.7% (95% CI, 7.4% to 17.0%)
 - (OR, 1.3; 95% CI, 0.7% to 2.3%).
- ***“One in 7 patients with first-ever acute ischemic stroke will test positive for one of the inherited thrombophilias, but the relation is likely to be coincidental rather than causal in almost all cases, irrespective of the pathogenic subtype of the ischemic stroke.”***
 - Hankey GJ, et al. Stroke. 2001 Aug;32(8):1793-9.

