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UNIVERSITY OF UTAH
SCHOOL OF MEDICINE
Department of Pathology

Inherited Thrombophilia Testing

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Prevalence and risk associated with inherited thrombotic disorders

Inherited Risk Factor	% General Population	% Patients w/ Thrombosis	Odds Ratio
Factor V Leiden	5	20	7/80
Elevated factor VIII	11	25	5–6
Homocysteinemia	5	10	2.6
Prothrombin mutation	2	6	2.8
Protein S deficiency	0.3	1–2	10
Protein C deficiency	0.3	3	10
Antithrombin deficiency	0.02	1	20

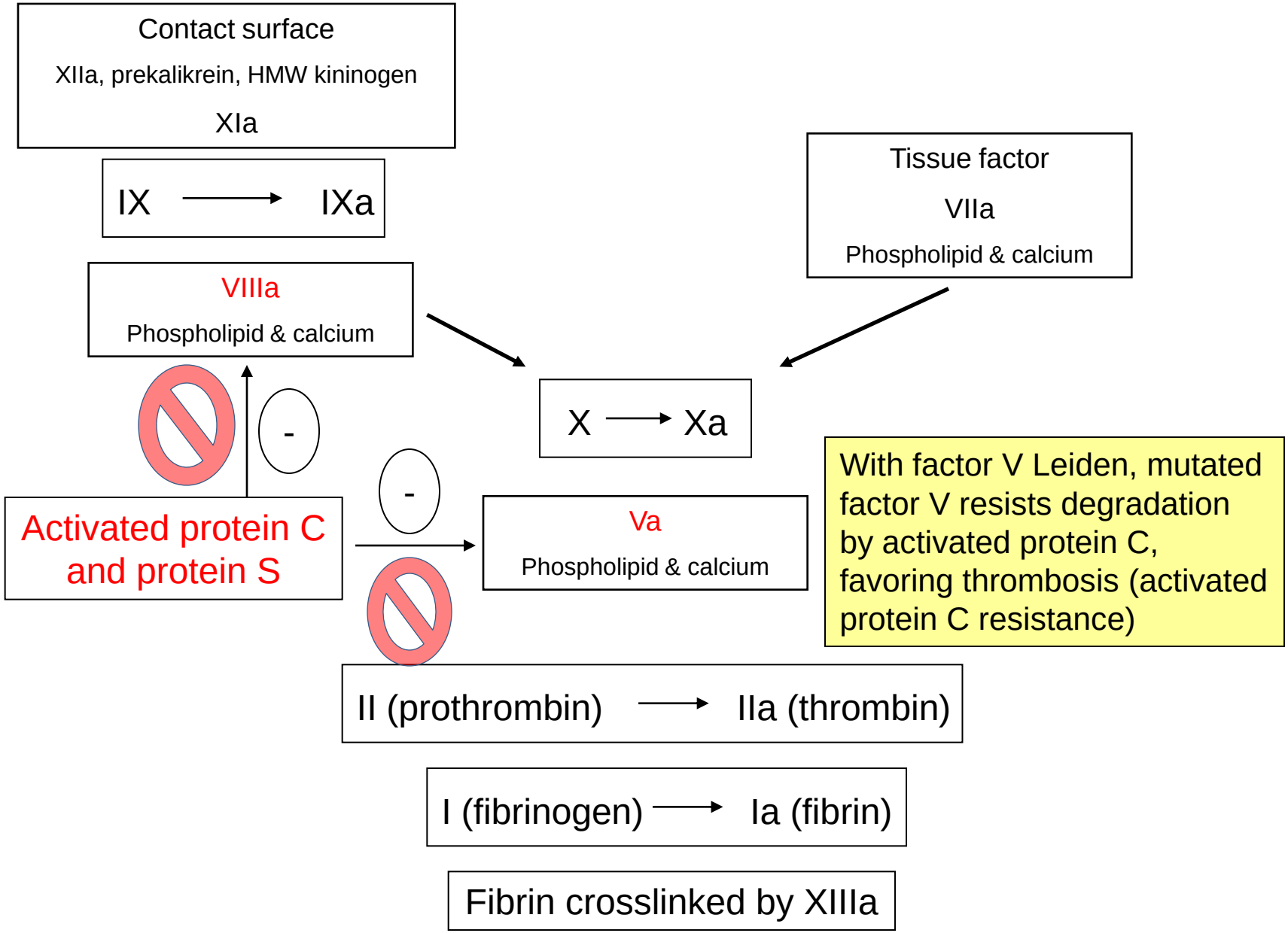
From Rosendaal FR. **Lancet** 353: 1167, 1999

Kearon C, *et al*. **Ann Rev Med** 51: 169, 2000

Risk is compounded if multiple abnormalities present

Recommended tests

- Activated protein C resistance profile with reflex to factor V Leiden
- Prothrombin (F2) G20210A mutation
- Protein C, functional
- Protein S, free
- Antithrombin activity


Contact surface
XIIa, prekalikrein, HMW kininogen
XIa
IX ⟶ IXa
Tissue factor
VIIa
Phospholipid & calcium
VIIIa
Phospholipid & calcium
X ⟶ Xa
-
Activated protein C and protein S
-
Va
Phospholipid & calcium
With factor V Leiden, mutated factor V resists degradation by activated protein C, favoring thrombosis (activated protein C resistance)
II (prothrombin) ⟶ IIa (thrombin)
I (fibrinogen) ⟶ Ia (fibrin)
Fibrin crosslinked by XIIIa

APC resistance test

	Normal patient	APC resistant patient
Clotting time with APC	65 seconds	32 seconds
Clotting time without APC	20 seconds	23 seconds

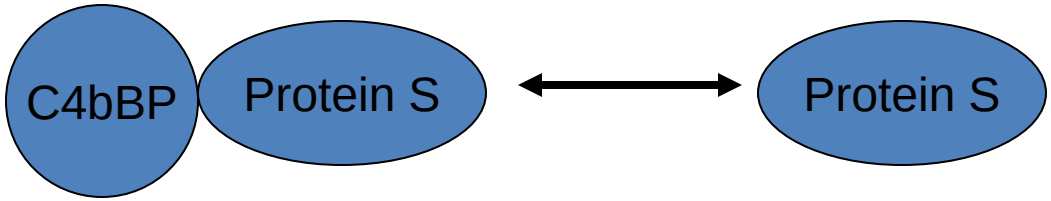
*This is the first-line test to detect most patients with FV Leiden mutations

*Confirm positives with DNA testing (identify heterozygotes vs. homozygotes)

- APC resistance reflex to FV Leiden is the recommended test

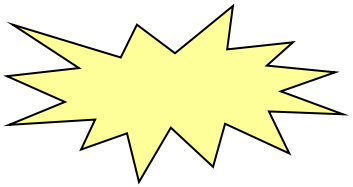
*APC assay is designed to minimize interference from anticoagulants

Binding protein



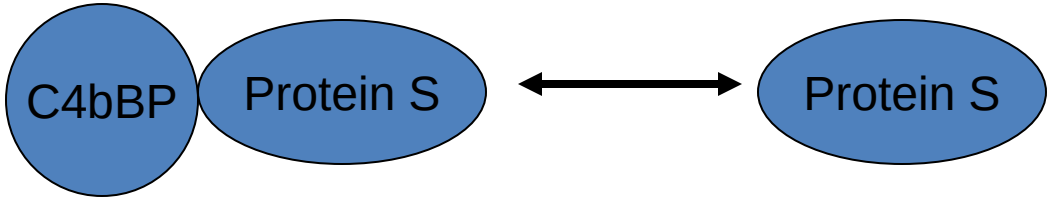
Bound protein S ~60%

Free protein S ~40%
Functional component



Stress, inflammation, pregnancy, oral contraceptives, etc.

Increased binding protein



Bound protein S increased

Free protein S decreased
Functional protein S decreased

Protein S testing is not reliable in conditions where the binding protein is increased

Problems with tests for antithrombin, protein C, and protein S

- Physiologic states associated with variable levels of AT, PC, and PS:

Pregnancy	↓AT, ↑PC, ↓↓ PS
Age	adult PC levels not reached until age 18
Gender	females have lower PS levels than males

Problems with tests for antithrombin, protein C, and protein S

- Pathologic states associated with variable levels of AT, PC, and PS:

Acute thrombosis	↓AT, ↓ PC, ↓ PS
Liver disease	↓AT, ↓ PC, ↓ PS
Nephrotic syndrome	↓AT, ↑ PC, ↓ PS
Warfarin	± ↑ AT, ↓ PC, ↓ PS
Heparin	↓ AT, interferes with functional assays for PC/PS

Guidelines for laboratory evaluation of inherited thrombosis

- Do not evaluate patients at the time of acute thrombosis*
- Avoid testing patients while they are taking anticoagulants*
- Postpone evaluation of pregnant patients until at least 1 month postpartum*
- Restrict testing to those likely to have an inherited disorder (younger patients, positive family history, recurrent clots)
- Use functional assays for AT, PC, and PS (or free PS levels)
- Repeat abnormal tests before making a definitive diagnosis of inherited thrombophilia
  - Abnormal results could be caused by patient condition/biologic variability, medications, or test variability

*Exceptions: DNA assays; homocysteine assay

ROLE OF INHERITED THROMBOPHILIA TESTING IN TREATMENT OF VENOUS THROMBOEMBOLISM

- Guideline recommendations are to anticoagulate most VTE patients 3-12 months.
- Despite such treatment, patients with idiopathic clots will recur at a rate of 10% per year.
- Inherited thrombophilia testing was originally thought to be useful in identifying the “higher-risk” patients who would recur, thus identifying a group of patients who would benefit from long-term therapy.

PREVALENCE AND RISK ASSOCIATED WITH INHERITED THROMBOTIC DISORDERS

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LABORATORY TESTING FOR INHERITED THROMBOPHILIA DOES NOT PREDICT RECURRENT THROMBOSIS

- Baglin et al. Lancet 362:523, 2003.
- Christiansen et al. JAMA 293:2352, 2005.

THE ROLE OF A THROMBOPHILIA DIAGNOSIS IN PREDICTING RECURRENT VENOUS THROMBOSIS

- Netherlands study – 474 DVT patients (1988–2000)
- Lab data analyzed: levels of AT, PC, PS, homocysteine, as well as factors VIII, IX, XI
- Clinical factors analyzed: gender, idiopathic vs provoked DVT, hormone use

Christiansen *et al*. **JAMA** 293:2352, 2005

THE ROLE OF A THROMBOPHILIA DIAGNOSIS IN PREDICTING RECURRENT VENOUS THROMBOSIS

RESULTS

- Recurrent clot occurred in 90 patients over 12 years.
- High-risk predictors: male gender, idiopathic clot, hormone use.
- The presence of a laboratory-diagnosed thrombophilic condition did not play a major role in predicting future clots.
- Clinical factors are more important than laboratory abnormalities in determining duration of anticoagulant therapy.

Christiansen *et al*. **JAMA** 293:2352, 2005

Journal of Thrombosis and Haemostasis, **6**: 1474–1477 DOI: 10.1111/j.1538-7836.2008.03055.x

ORIGINAL ARTICLE

Testing for inherited thrombophilia does not reduce the recurrence of venous thrombosis

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Hypothesis: Patients with a first venous thrombosis who are tested for inherited thrombophilia have a reduced risk of VT recurrence because of change in clinical management.

Results: Tested patients did not have a different recurrence risk from non-tested patients.

Conclusion: Thrombophilia tests are regularly performed, but results are not often used in patient management.

ANALYSIS OF PHYSICIAN TEST ORDERING PRACTICES OF SAMPLES REFERRED TO ARUP LABORATORIES

- A 2002 CAP Thrombophilia Conference reported evidence–based recommendations for thrombophilia testing
- To determine the extent of acceptance of these recommendations, a retrospective analysis of laboratory orders and test results was performed from samples referred to ARUP Laboratories for thrombophilia testing between Sept 2005 through Aug 2006

Jackson BR *et al*. **BMC Clin Pathol** 8:3, 2008

METHODS OF ANALYSIS OF TEST ORDERS AND RESULTS

- Results of assays for protein C, protein S, antithrombin, factor V Leiden (DNA and APC-R assays), and the prothrombin mutation were obtained (Sept 2005 – Aug 2006).
- Total test orders = 197,771
- Relative to ordering volumes were determined, as was the ratio of activity to antigen assays (e.g., PC activity vs PC antigen).
- The ratio of APC-R assays to DNA tests ordered for factor V Leiden was determined.
- The proportion of functional clot-based assays for PC, PS, and AT ordered on samples with elevated PT values was determined.

Jackson BR *et al*. **BMC Clin Pathol** 8:3, 2008

POSITIVITY RATES BY TEST ORDERED

Tests	Positivity rates (%)	Fraction of positive with INR > 1.3 (%)
PS functional	**17.7**	**21.2**
PS total antigen	**4.8**	**40.5**
PS free antigen	**18.5**	**25.0**
PC functional	**13.7**	**53.8**
PC total antigen	**12.9**	**33.3**

For samples on which PC, PS, and AT were all ordered, 15.7% of samples had positive results for 2 of 3 analytes

Jackson BR *et al*. **BMC Clin Pathol** 8:3, 2008

CONCLUSIONS FROM THE THROMBOPHILIA TEST SURVEY

- Between 20 – 50% of samples with low PS or PC levels also had an INR > 1.3
- The financial implications of inappropriate thrombophilia testing are considerable
- Implications for patient safety

DR. RODGER'S GUIDELINES

- Patients are encouraged **not** to undergo routine thrombophilia testing unless they have female siblings and/or children at risk for whom a thrombophilia diagnosis will change management.
- Patients are tested for antiphospholipid antibodies because positive results will lead to longer-term anticoagulation.
- Patients ≥ 40 years of age are encouraged to undergo routine recommended cancer screening.
- Testing for AT, PC, and PS can be considered if done at a time distant from the acute event and off anticoagulant therapy.

PREDICTIVE VALUE OF D-DIMER TEST FOR RECURRENT VTE AFTER ANTICOAGULATION WITHDRAWAL

Palareti *et al*. **Circulation** 108:313,2003.

- 599 patients were treated for VTE and prospectively followed with D-dimer levels after anticoagulation was discontinued.
- 37% of patients had increased D-dimer levels; this was associated with increased VTE recurrence in all subgroups (idiopathic, provoked, thrombophilia, or no thrombophilia).
- Negative predictive value = 94%.