Optimal Utilization of Thrombophilia Testing

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Disclosure (s)

• Relevant financial relationship(s)
  • None

• Off-label usage
  • None
Learning Objectives

• Explain the concept of thrombophilia
• Recognize the congenital and acquired thrombophilias
• State the practical application of thrombophilia in patient management
• Understand limitations of selected assays
• Realize the value of algorithmic approach to testing
Multifactorial Disease

Acquired + inherited

Thrombosis

Acquired

Inherited

Acquired + acquired

Inherited + inherited
Thrombophilia

**Hereditary**

Coagulopathy

- Activated protein C resistance
  - Factor V Leiden
- Prothrombin G20210A
- Selected dysfibrinogenemia variants
- Antithrombin
- Protein C
- Protein S

**Acquired**

Coagulopathy

- Antiphospholipid antibodies
  - Lupus anticoagulant
  - Anti-cardiolipin
  - Anti-beta-2 GP I

Clinical Risk factors
- Numerous
Acquired Clinical Risk Factors for VTE
Nested Case-Control Study (625 Case-Control Pairs)

- Surgery
- Trauma
- Inpatient
- Malignancy with chemotherapy
- Malignancy without chemotherapy
- Central venous catheter or pacemaker
- Neurologic disease
- Superficial vein thrombosis
- Varicose veins/age 45 yr
- Varicose veins/age 60 yr
- Varicose veins/age 70 yr
- CHF, VTE incidental on autopsy
- CHF, antemortem VTE/causal for death
- Liver disease

• Pre-analytical:
  • Patient selection
• Analytical (laboratory aspects):
  • Types and sequence of testing
  • Influence of anticoagulants
• Post analytical:
  • Application to patient care
Patient Population

• Asymptomatic individual

General (population)screening **NOT indicated**

Anticipated exposure to an acquired thrombophilia
Hospitalization, surgery, pregnancy, orthopedic surgery
oral contraceptive (OCP), hormone replacement (HRT)
### Odds Ratio for Risk of VTE in Orthopedic Surgery

<table>
<thead>
<tr>
<th>Study or Subcategory</th>
<th>No VTE events n/N</th>
<th>VTE events n/N</th>
<th>OR (random) 95% CI</th>
<th>OR (random) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Philipp et al</td>
<td>3 / 55</td>
<td>2 / 30</td>
<td>1.24 (0.20-7.85)</td>
<td></td>
</tr>
<tr>
<td>Woolson et al</td>
<td>2 / 45</td>
<td>3 / 36</td>
<td>1.95 (0.31-12.38)</td>
<td></td>
</tr>
<tr>
<td>Lowe et al</td>
<td>7 / 240</td>
<td>7 / 116</td>
<td>2.14 (0.73-6.24)</td>
<td></td>
</tr>
<tr>
<td>Svenson et al</td>
<td>13 / 141</td>
<td>9 / 57</td>
<td>1.85 (0.74-4.60)</td>
<td></td>
</tr>
<tr>
<td>Lindahl et al</td>
<td>82 / 625</td>
<td>9 / 20</td>
<td>5.42 (2.18-13.47)</td>
<td></td>
</tr>
<tr>
<td>Ryan et al</td>
<td>22 / 613</td>
<td>10 / 212</td>
<td>1.33 (0.62-2.86)</td>
<td></td>
</tr>
<tr>
<td>Wahlander et al</td>
<td>48 / 932</td>
<td>23 / 323</td>
<td>1.41 (0.84-2.36)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>2,651</td>
<td>794</td>
<td>1.86 (1.27-2.74)</td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: $\chi^2=7.38$, df=6 (P=0.29), $I^2=18.7%$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $z=3.16$ (P=0.002)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prothrombin G202/OA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Westrich et al</td>
<td>0 / 14</td>
<td>4 / 14</td>
<td>12.43 (0.60-256.66)</td>
<td></td>
</tr>
<tr>
<td>Wahlander et al</td>
<td>25 / 932</td>
<td>9 / 323</td>
<td>1.04 (0.48-2.25)</td>
<td></td>
</tr>
<tr>
<td>Subtotal (95% CI)</td>
<td>946</td>
<td>337</td>
<td>2.33 (0.23-23.56)</td>
<td></td>
</tr>
<tr>
<td>Test for heterogeneity: $\chi^2=2.50$, df=1 (P=0.11), $I^2=60.1%$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Test for overall effect: $z=0.72$ (P=0.47)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## Cost effectiveness of testing for factor V Leiden (FVL)

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Odds ratio for VTE</th>
<th>Absolute risk VTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral contraceptives (OCP)</td>
<td>3.10 (2.17 to 4.42)</td>
<td>300 per 100,000 women-yrs (0.3% per woman-yr)</td>
</tr>
<tr>
<td>FV Leiden</td>
<td>3.78 (2.22 to 6.42)</td>
<td></td>
</tr>
<tr>
<td>OCP + FVL</td>
<td>15.62 (8.66 to 28.15)</td>
<td></td>
</tr>
</tbody>
</table>

## Cost effectiveness of testing for factor V Leiden (FVL)

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Odds ratio for VTE</th>
<th>Absolute risk VTE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone replacement Therapy</td>
<td>3.16 (1.9 to 5.23)</td>
<td></td>
</tr>
<tr>
<td>FV Leiden</td>
<td>3.58 (1.43 to 8.97)</td>
<td></td>
</tr>
<tr>
<td>HRT + FVL</td>
<td>13.15 (4.28 to 40.47)</td>
<td>900 to 1800 per 100,000 women- yrs (1 to 2% per woman-yr)</td>
</tr>
</tbody>
</table>

Cost effectiveness of FVL testing in asymptomatic patients

• Not cost effective:
  • Pre-orthopedic surgery
  • Pre-oral contraceptive prescription

• Possible cost effective (in U.K.)
  • Pre-HRT prescription

• Studies on other thrombophilias not available
Patient Population

• Asymptomatic individual

  General (population)screening **NOT indicated**

  Anticipated exposure to an acquired thrombophilia
  Hospitalization, surgery, pregnancy, orthopedic surgery
  oral contraceptive (OCP), hormone replacement (HRT)

• **Symptomatic** patient with thromboembolism
Patient selection: A suggested approach

Arterial thrombosis

- Antiphospholipid Antibodies
- Dysfibrinogenemia etc

Venous thrombosis

- Temporary risk factor

- Testing not indicated
ASH Choosing wisely campaign: duration of anticoagulation

Recommendations

…do not order thrombophilia testing for VTE occurring in association with a transient VTE risk factor (surgery, trauma, prolonged immobility)…

…do not treat with an anticoagulant for >3 months in a first VTE occurring in association with a transient risk factor…

Patient selection

**Arterial thrombosis**
- Antiphospholipid antibodies

**Venous thrombosis**
- Temporary risk factor
- Idiopathic with or without family history

Testing:
- Not indicated
- Possibly indicated
• Pre-analytical:
  • Patient selection
• Analytical (laboratory aspects):
  • Types and sequence of testing
  • Influence of anticoagulants
• Post analytical:
  • Application to patient care
# Thrombophilia markers

<table>
<thead>
<tr>
<th>Good evidence</th>
<th>Weak Evidence</th>
<th>Lack of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>High TAFI</td>
<td>Plasminogen deficiency</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>Elevated fibrinogen, factor IX and factor XI</td>
<td>High PAI-1 levels</td>
</tr>
<tr>
<td>AT/PC/PS deficiency</td>
<td>EPCR polymorphism</td>
<td>Factor XIII Leu34Val</td>
</tr>
<tr>
<td>Non-O blood group</td>
<td></td>
<td>Lp(a)</td>
</tr>
<tr>
<td>High factor VIII</td>
<td></td>
<td>MTHFR (677 and 1298)</td>
</tr>
<tr>
<td>Dysfibrinogenemia</td>
<td></td>
<td>Thrombomodulin/ACE/PZ polymorphism</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td></td>
<td>ADAMTS 13 polymorphism</td>
</tr>
</tbody>
</table>

Franchini et al Thromb Haem 2016; 115:25-30
Thrombophilia Profile

Testing begins with:
- Prothrombin Time (PT), Plasma
- Activated Partial Thromboplastin Time (APTT), Plasma
- Dilute Russell's Viper Venom Time (DRVVT), Plasma
- Thrombin Time (Bovine), Plasma
- Fibrinogen, Plasma
- D-Dimer, Plasma
- Soluble Fibrin Monomer
- Antithrombin Activity, Plasma
- Protein C Activity, Plasma
- Protein S Antigen, Free, Plasma
- Prothrombin G20210A A Mutation, Blood
- Activated Protein C Resistance V (APCRV), Plasma
- Special Coagulation Interpretation

All initial testing within reference ranges for age and gender:
- No evidence of thrombotic diathesis
- No further testing is performed

Antithrombin Activity:
- <80%
- No evidence of an acquired deficiency

PT: ≥14.0 seconds
- PT Mix 1:1
  - ≥14.0
  - <14.0

APTT: >36 seconds
- APTT Mix 1:1
  - ≤36
  - >36

DRVVT: ≥1.2 seconds
- DRVVT Mix 1:1
  - ≥1.2
  - <1.2

Thrombin Time (Bovine):
- Normal – no evidence of heparin or dys/hypofibrinogenemia
- Repilase Time
- Factor V Leiden (R506Q) Mutation
- Protein C Activity: <70%
- Protein C Antigen
- Protein S Antigen, Free
  - Males: <65%
  - Females: <50 years: <50%, ≥50 years: <65%

APCRV:
- <2.3
- OR
- Prolonged baseline APTT

Protein S Antigen, Total
- Males: <65%
- Females: <50 years: <50%, ≥50 years: <65%

Evidence of inhibition
- Evidence of coagulation factor deficiency

Evidence of inhibition
- Evidence of coagulation factor deficiency

No evidence of heparin in sample
- Platelet neutralization procedure (PNP)
  - Does not shorten
  - Shortens by 4-5 seconds

Possible factor inhibitor
- Evidence of lupus-like anticoagulant
  - No diagnostic of lupus-like anticoagulant

Possible dys/hypofibrinogenemia
- Anticoagulant effect

Unfractionated/low-molecular weight heparin or direct thrombin inhibitor (e.g., dabigatran, argatroban)

An interpretive report is provided that includes all profile tests (always performed) and any reflex tests performed (if appropriate).
Profile versus single order

- Known family history of a thrombophilia
  - reasonable to perform focused testing
- No known personal or family history of thrombophilia
  - reasonable to take a profile approach
Timing of thrombophilia testing
Phases of anticoagulation

Initial Rx
0-7 days
Parenteral
VKA or other agent
No effect of thrombophilia

Acute VTE Rx
3 months
Multifactorial effect

Long term
3 months

Extended
> 3 months
Secondary prophylaxis of VTE

Client analysis

Kearon C et al Chest 2012;141 (2)(Suppl)e419S-e494S
Activated Protein C Resistance and Factor V Leiden

• Most common congenital hereditary thrombophilia among whites

• Protein phenotype
  • Normally: Activated protein C (APC) inactivates activated factor V (fVa)
  • APC resistance: Mutated factor V resists inactivation by APC

• Genetic basis
  • Factor V Leiden (R506Q) mutation

• Testing strategy
  • Initial APC-R assay, FV Leiden only if indicated
Screening With the Activated Protein C Resistance Assay Yields Significant Savings in a Patient Population With Low Prevalence of Factor V Leiden

Laura J. Taylor, MT(ASCP), Robert A. Oster, PhD, George A. Fritsma, MS, MT(ASCP), Patricia H. Tichenor, MT(ASCP), Cari E. Reed, MT(ASCP), Barbara M. Eiland, MT(ASCP), Christine L. Hudson, MT(ASCP), and Marisa B. Marques, MD

Key Words: Factor V Leiden; Activated protein C resistance; Cost savings

DOI: 10.1309/4370VLY9P8DDEWF6
Mayo Clinic, Rochester experience vs Optum labs

• Optum labs data warehouse
  • >100 million enrollees
  • Medical claims data for laboratory testing etc
• Inpatient and outpatient
Mayo APCR/FV Leiden vs Optum Labs database

<table>
<thead>
<tr>
<th>Test description</th>
<th>Mayo Sp Coag Lab</th>
<th>Optum Labs</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC-R</td>
<td>1256</td>
<td>5,395</td>
</tr>
<tr>
<td>FV Leiden</td>
<td>268</td>
<td>80,129</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(78,525)</td>
</tr>
<tr>
<td>Ratio: APCR:FVL</td>
<td>~1: 0.2</td>
<td>~1:15</td>
</tr>
<tr>
<td>Cost per evaluated individual</td>
<td>$36.38 (savings: $47.39)</td>
<td>$83.77</td>
</tr>
</tbody>
</table>
# ECAT 2016: APCR Normal control plasma sample

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Assigned value</th>
<th>CV(%)</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total group</td>
<td>178</td>
<td>(Varied with kit)</td>
<td>33.1</td>
<td>0.76-5.90</td>
</tr>
</tbody>
</table>

### Classification

<table>
<thead>
<tr>
<th>Classification</th>
<th>Normal</th>
<th>Borderline</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>175</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

### Secondary classification

<table>
<thead>
<tr>
<th>Secondary classification</th>
<th>Homozygous FVL</th>
<th>Heterozygous FVL</th>
<th>Non-conclusive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>
**ECAT 2016: APCR Heterozygous FVL plasma sample**

<table>
<thead>
<tr>
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<th>Assigned value</th>
<th>CV(%)</th>
<th>range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total group</td>
<td>178</td>
<td>Varied with kit</td>
<td>14.8</td>
<td>0.62-2.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>Classification</th>
<th>Normal</th>
<th>Borderline</th>
<th>Abnormal</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1</td>
<td>1</td>
<td>195</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Secondary classification</th>
<th>Homozygous FVL</th>
<th>Heterozygous FVL</th>
<th>Non-conclusive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>9</td>
<td>117</td>
<td>24</td>
<td></td>
</tr>
</tbody>
</table>

### Diagram

- **Ratio vs. Count**
  - X-axis: Ratio (0.2, 0.5, 0.9, 1.3, 1.7, 2.1, 2.5, >2.8)
  - Y-axis: Count (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100)
  - Bars represent different ratio ranges.
Protein C deficiency: assays & variables

- Chromogenic assay: miss 1 to 2% of deficiencies
- Clotting based assay: miss ~1% of deficiencies
- Antigen assays: miss 14% of deficiencies
  - Miss type 2 deficiency
- Variables:
  - Vitamin K dependent
  - False increase with anti-Xa/Direct thrombin inhibitors
Limitations of PS activity assays

- PS activity measurements in normals:
  - Levels reduced in 10 to 15% normal donors
    - Upon recheck-levels returns to normal
  - Subject to technical limitations
    - Measuring a cofactor function
    - Significantly affected by biological and analytical variables

Reference ranges: lab established vs manufacturer provided information

<table>
<thead>
<tr>
<th>Assay</th>
<th>% below established reference range</th>
<th>% below manufacturer’s reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free PS Ag</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Kit A PS activity</td>
<td>11%</td>
<td>13%</td>
</tr>
<tr>
<td>Kit B PS activity</td>
<td>18%</td>
<td>38%</td>
</tr>
<tr>
<td>Kit C PS activity</td>
<td>24%</td>
<td>Not available</td>
</tr>
<tr>
<td>Kit D PS activity</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>Total PS Ag</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

UK NEQUAS: normal protein S plasma

Number of participants

Protein S activity u/dL

Kit A
Kit B
Kit C
Kit D

Walker, ID & Jennings, I. Quality in Laboratory Hemost & Thromb. 2nd edition
### ECAT Proficiency testing: PS deficiency sample (2015-3)

<table>
<thead>
<tr>
<th>Total Group</th>
<th>Assigned value</th>
<th>Range of results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratories (5 different kits)</td>
<td>34%</td>
<td>21-111</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Classification</th>
<th>Normal</th>
<th>Borderline normal</th>
<th>Borderline abnormal</th>
<th>Abnormal</th>
<th>No classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>147</td>
<td>2</td>
</tr>
</tbody>
</table>

![Histogram of results]
Limitations of PS activity assays

- Measurement of a cofactor activity
- Influenced by different biological and preanalytical variables.
- Interferences:
  - Artifactual elevation of PS activity:
    - Lupus anticoagulants
  - Artifactual reduction of PS activity
    - Elevated factor VIII:C
    - Factor V Leiden mutation (selected assays)

• Pre-analytical:
  • Patient selection

• Analytical (laboratory aspects):
  • Types and sequence of testing
  • Influence of anticoagulants

• Post analytical:
  • Application to patient care
Risk of Recurrent VTE & Factor V Leiden

Incidence of recurrent thromboembolism (%)

Follow-up (yr)

Simioni P: NEJM 336:399, 1997
Risk of Recurrent VTE & Factor V Leiden

Follow-up (mo)

- Noncarrier
- Heterozygotes
- Homozygotes

Lindmaker P: Throm & Haem 81:684, 1999
## Risk of recurrent VTE

<table>
<thead>
<tr>
<th>Recurrence % per yr (CI)</th>
<th>PS (53)</th>
<th>PC (52)</th>
<th>AT (25)</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8.4 (5.8-11.4)</td>
<td>6.0 (3.0-8.7)</td>
<td>10 (6.1-15.5)</td>
<td>7.7 (6.1-9.5)</td>
</tr>
</tbody>
</table>

### Cumulative recurrence rates (16%)

<table>
<thead>
<tr>
<th>Year</th>
<th>PS</th>
<th>PC</th>
<th>AT</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 yr</td>
<td>16</td>
<td>10</td>
<td>24</td>
<td>15</td>
</tr>
<tr>
<td>5 yr</td>
<td>44</td>
<td>37</td>
<td>49</td>
<td>42</td>
</tr>
<tr>
<td>10 yr</td>
<td>60</td>
<td>39</td>
<td>59</td>
<td>53</td>
</tr>
<tr>
<td>15 yr</td>
<td>77</td>
<td>59</td>
<td>77</td>
<td>68</td>
</tr>
</tbody>
</table>

PS: protein S, PC: protein C; AT: antithrombin; yr: year

Brouwer J-LP et al. Thrombosis & Haemostasis 2009101;93
### VTE and thrombophilia

<table>
<thead>
<tr>
<th>Condition</th>
<th>Relative Risk incident(^1) VTE (95% CI)</th>
<th>Relative Risk recurrent VTE (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated protein C resistance (factor V Leiden)</td>
<td>4.3 (1.9 – 9.7)</td>
<td>1.3 (1.0-3.3)</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>1.9 (0.9-4.1)</td>
<td>1.4 (0.9-2.0)</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>11.3 (5.7-22.3)</td>
<td>2.5</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>17.5 (9.1-33.8)</td>
<td>2.5</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>32.4 (16.7-62.9)</td>
<td>2.5</td>
</tr>
<tr>
<td>Non-O blood group</td>
<td>1.79 (1.56-2.05)</td>
<td>2.0 (1.2-3.8)</td>
</tr>
</tbody>
</table>
Conclusions: Optimal Utilization of Thrombophilia testing

• Optimal utilization begins with patient selection
• Know the situations where testing is NOT indicated
• Judicious ordering of Thrombophilia testing if it affects patient management
• Know the laboratory variables that affect results
• Algorithmic approach within thrombophilia profiles provides the most cost effective approach to testing