Thrombophilia Testing

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Thrombophilia-Not all the same

• High risk thrombophilia
  – Antithrombin deficiency - 1.8 % per year (95% CI 1.1-2.6%)
  – Protein C deficiency - 1.5% per year (1.1-2.1%)
  – Protein S deficiency - 1.9% per year (1.3-2.6%)

• Moderate risk thrombophilia
  – Factor V Leiden - 0.5% per year (0.4-0.6%)
  – Prothrombin gene mutation - 0.3% per year (0.2-0.5%)
  – Factor VIII - 0.5% per year (0.4-0.5%)

• Low risk thrombophilia
  – Factor IX - 0.1% per year (0.02-0.2%)
  – Factor XI - 0.2% per year (0.06-0.6%)
  – Hyperhomocysteinemia – 0.1% per year (0.05-0.3%)

Lijfering WM et al. Blood 2009
Antithrombin (III) Deficiency

- First proposed to exist by Morawitz in 1905
- Binds an inactivates IIa, Xa, IXa and Xla.
- Accelerated 1000X in presence of heparin
- First clinical description: 1965 by Egeberg
- Prevalence- 1 per 2-5,000
- Autosomal dominant inheritance
- Increases risk of venous thrombosis by 20-50-fold
- Thrombosis- 90% venous, 60% precipitated
Antithrombin Deficiency

- **Inherited**
  - Type I (Quantitative deficiency)- decreased protein levels, decreased activity
  - Type 2a (Qualitative deficiency)- normal protein levels, decreased activity (active site mutations)
  - Type 2b (Qualitative deficiency)- normal protein levels, decreased heparin binding (heparin binding site mutations)

- **Acquired**
  - Neonatal period
  - Nephrotic syndrome
  - Acute Thrombosis
  - DIC
  - Liver disease
  - L-asparaginase/heparin therapy

- **Diagnosis**
  - Use activity assay for diagnosis
  - Avoid situations associated with acquired deficiency
Antithrombin-Heparin Cofactor Assay

Normal range 80 - 120%

Bilirubin > 20mg/dl interfere with assay
AT antigen assay

Normal range 68 - 128%

Lipemia, cloudy specimens and RF may affect assay
Protein C deficiency

- Identified by Mammen et al in 1960
- Stenflo et al. designated it protein C in 1976
- Vitamin K dependent protease
- First clinical description - Griffin, 1981
- Prevalence - 1/500
- Autosomal dominant inheritance
- Increases thrombosis risk ~ 10-20 fold
- Primarily venous thrombosis, 70% spontaneous events
Protein C deficiency

- Inherited
  - Type I: low antigen, low activity
  - Type II: normal antigen, low anticoagulant activity

- Acquired
  - Vitamin K deficiency
  - Warfarin therapy
  - Acute thrombosis (?)
  - Pregnancy/Estrogen
  - Inflammation

- Diagnosis
  - Use an activity assay
  - Measure PT at same time
  - Avoid situations associated with acquired deficiency
Protein C activity assays

Agkistrodon contortrix venom

Normal Range 65 - 140%, affected by heparin > 1U/ml, warfarin
Protein C antigen assays

Normal range 60-150%

May be affected by anti-rabbit antibodies
Protein S deficiency

- Discovered by Davie et al. in Seattle, 1977
- Walker identified function as a cofactor for protein C in 1980.
- Requires vitamin K dependent Gla domains for activity
- Prevalence- 1/500-1/1000
- Autosomal dominant inheritance
- Increases the risk of thrombosis 8-10-fold
- Presentation- 90% venous thrombosis, 60% spontaneous
Protein S function
Protein S deficiency

• Inherited deficiency
  – Type I - low antigen, low activity
  – Type IIa - normal antigen, low activity
  – Type IIa - normal total antigen, low free antigen, low activity

• Diagnosis
  – Use activity assay for screening
  – Do not measure in situations associated with acquired deficiency

• Acquired deficiency
  – Vitamin K deficiency
  – Liver disease
  – Warfarin therapy
  – DIC/thrombosis (?)
  – Estrogen/pregnancy
  – Inflammation
  – L-asparaginase
Protein S Activity Assays

Normal range: 65% - 140%
Affected by heparin > 1.2 U/ml, lupus inhibitors

Protein S level

Phospholipids

aPTT

APC

Xa
Protein S Antigen assays

Normal range- 60% - 150%
Anti-mouse antibodies can affect results

O-phenylenediamine

Peroxidase

Absorbance at 450 nM

Protein S antigen
Free Protein S antigen assays

Absorbance at 450 nM

Free Protein S antigen
Factor V Leiden

• Activated protein C resistance phenotype identified - Björn Dahlbäck-1993
• Factor V mutation identified- Dahlbäck B, et al. 1994; Bertina R et al.
• Prevalence
  – European-Americans
    Heterozygotes 5%
    Homozygotes 0.02%
  – Hispanic Americans- 2.2%
  – African- Americans- 1.2%
  – Native Americans- 1.2%
  – Asian Americans- 0.5%
  – Native Africans, Asians- very low
Factor V Leiden

- Prevalence
  - Unselected patients - 20%
  - Selected patients - 40%

- Thrombosis incidence-
  - FVL +/- = 0.2-0.5%/year
  - FVL+/+ = 1-2.3%/year

- Thrombosis risk increased by...
  - Estrogens
  - Pregnancy
  - Cancer
  - Surgery

306 506 679

Factor Va

S

APC
Factor V: A multi-functional coagulation factor

IIa

Factor Va

Factor V

Factor Vac

FVIIIla

FVIIIi

APC

S
APC resistance assay

APC ratio = \frac{70 \text{ sec}}{30 \text{ sec}} = 2.33

APC ratio = \frac{48 \text{ sec}}{30 \text{ sec}} = 1.6
Prothrombin G20210A mutation

- Prevalence: 1-2%
- Autosomal dominant
- Increased prothrombin levels
- VTE risk $\uparrow$ 2.8X
Other inherited hypercoagulable conditions

• Elevated Factor VIII levels (> 95 percentile)
  – Increase RR of first and recurrent DVT/PE 3-5-fold
  – Thrombosis/Inflammation can increase levels
  – Measure activity assay 6 months after thrombotic event

• Dysfibrinogenemia
  – Rare cause of DVT/PE
  – Use fibrinogen activity and antigen levels to screen
Antiphospholipid Antibodies

- Present in 8.5 to 14% of VTE pts., 30-40% of SLE, 10% of FWS
- Associated with venous and arterial disease, abortion and thrombocytopenia
- Occur in autoimmune disease, tumors, infections, drugs (procainamide, quinidine, phenothiazines) and primary disorder
Antiphospholipid Antibodies

- Thrombosis associated with high titer ACL (>40 GPL), LA>ACL, SLE- or primary syndrome
- Tests - Dilute Russell Viper venom time, High-sensitivity aPTT, ELISA for IgG, IgA and IgM antibodies
- Why thrombosis? - endothelial damage, complement activation, tissue factor expression, ↓ protein C activation, ↓ protein S
- Recurrent thrombosis rate 20-50% over 2 years
Dilute Russell viper venom time

Confirm Ratio

\[
\frac{\text{dRVVT (sec)}}{\text{dRVVT + PL (sec)}} = \frac{60 \text{ sec.}}{30 \text{ sec.}} = 2
\]

Normal (1 - 1.4)
Testing for anti-phospholipid antibody syndrome
Diagnostic criteria of APS

• Clinical criteria
  – Vascular thrombosis
  – Pregnancy morbidity
    • One or more unexplained fetal deaths, One or more premature births at or before 34th week, Three or more unexplained spontaneous abortions

• Laboratory criteria
  – Anticardiolipin antibodies (IgG or IgM in 40 PL units or higher)
  – Beta 2 Glycoprotein I abs (IgG or IgM ≥ 99th percentile)
  – Lupus anticoagulant (aPTT or dRVVT)
  – Confirmed positive tests 12 weeks later

Antiphospholipid syndrome is associated with recurrent thromboembolism

Reasons to do thrombophilia testing

- Identify the reason for a thrombotic episode
- Determine the duration of anticoagulation
- Identify a reason for adverse pregnancy outcomes (≥ 20 weeks)
- Determine management of thrombotic events during future pregnancies
Thrombophilia testing - What patients?

- Age < 50
- Family history of VTE
- VTE in unusual sites (Abdomen, CNS)
- Extensive VTE
- Idiopathic VTE
- Recurrent VTE
- Warfarin skin necrosis
- Autoimmune disorders
Thrombophilia Testing - Who gets what test?

• Venous Thromboembolism

• Arterial Thromboembolism
  – Antiphospholipid syndrome, dysfibrinogenemia
Thrombophilia testing - What? When?

• Factor V Leiden
  – Screen with APC resistance assay
    • Can be done on therapeutic doses of heparin or warfarin
  – Confirm results with DNA-based assay
    • Can be done on anticoagulation
  – Timing - during or after acute episode

• Prothrombin gene mutation
  – Use DNA-based assay
  – Test during or after acute episode

• MTHFR C677T genotype
  – DNA-based assay
  – Can test during or after acute episode
Thrombophilia testing - What? When?

• **Antithrombin deficiency**
  – Use AT activity assay
  – Alternative Causes
    • Acute thrombosis
    • Liver disease
    • Pregnancy, estrogens
    • Neonatal period
  – May test acutely but follow up abnormal results later

• **Protein C deficiency**
  – Use protein C activity assay
  – Alternative Causes
    • Acute Thrombosis
    • Vitamin K deficiency
    • Liver disease
    • Neonatal period
  – If positive, assess protein C antigen level
  – May test acutely but confirm if abnormal later off warfarin
Thrombophilia testing - What? When?

• Protein S activity
  – Use protein S activity assays
  – Alternative Causes
    • Vitamin K deficiency
    • Liver disease
    • DIC
    • Estrogen/pregnancy
    • Neonatal period
  – If positive, assess total and free protein S antigen
  – Test after acute episode, off warfarin
Do the Results of Thrombophilia Tests Help to Determine Duration of Therapy?

- **Baglin, 2003** (N=570) 24 mos.
  - Recurrent VTE: 15%
  - HR: 1.5 (0.8-2.8)

- **Christiansen, 2005** (N=474) 84 mos.
  - Recurrent VTE: 15%
  - HR: 1.4 (0.9-2.2)

- **Santamaria 2005** (N=267) 46 mos.
  - Recurrent VTE: 15%
  - HR: 1.8 (1-3.1)

- **Prandoni 2007** (N=1626) 50 mos.
  - Recurrent VTE: 15%
  - HR: 2.0 (1.5-2.7)
The problems with conventional thrombophilia testing

• Thrombophilia testing is strongly influenced by patient status
• Thrombophilia testing is not a global assessment of thrombotic risk
• Thrombophilia testing converts a continuous variable into a dichotomous variable
• Thrombophilia testing ignores the complications of anticoagulation
Duration of therapy: 2008 ACCP VTE guidelines

- VTE- transient risk factor- 3 months
- VTE- idiopathic- at least 3 months
- Distal DVT- 3 months
- Recurrent VTE- indefinite
- Cancer- indefinite or until malignancy resolved
  - Recommend LMWH for first 3-6 months of therapy
- Antiphospholipid syndrome – indefinite therapy
- Significant inherited thrombophilia – 6 months - indefinite

Kearon et al. Chest 2008
Reasons to do thrombophilia testing

• Identify the reason for a thrombotic episode
• Determine the duration of anticoagulation
• Determine treatment during pregnancy
• Identify a reason for adverse pregnancy outcomes
Characteristics of previous VTE influence pregnancy VTE risk

- Prospective study of 125 women with history of VTE
- No antepartum AC
- Baseline duplex US + duplex or V/Q for symptoms of VTE
- Post-partum AC – UFH 5000-7500 q12h + warfarin (INR 2-3) X 4-6 weeks
- Thrombophilia test- Positive in 25 (26%)
- VTE- 6/125 (4.8%)
  - 3 ante-partum (2 idiopathic, 1 OCP)
  - 3 post-partum (2 idiopathic, 1 C section)
  - no fatal events
- No bleeding complications
- Conclusion- Women with triggered VTE and no thrombophilia do not need antepartum AC

# Anticoagulant Regimens

<table>
<thead>
<tr>
<th>Anticoagulant regimen</th>
<th>Dose</th>
</tr>
</thead>
</table>
| **Prophylactic LMWH** | Dalteparin 5000 units sc q24h  
Enoxaparin 40 mg sc q24h  
Tinzaparin 4,500 units sc q24h |
| **Prophylactic UFH** | 1\textsuperscript{st} Trimester- 5000-7500 units sc q12h  
2\textsuperscript{nd} Trimester- 7500-10,000 units sc q12h  
3\textsuperscript{rd} Trimester- 10000 units sc q12h |
| **Intermediate dose LMWH** | Dalteparin 5000 units sc q12h  
Enoxaparin 40 mg sc q12h  
Tinzaparin 4,500 units sc q12h |
| **Intermediate dose UFH** | UFH sc q12h adjusted to anti-Xa of 0.1-0.3 units/mL |
| **Therapeutic dose LMWH** | Dalteparin 100 u/kg sc q12h or 200u/kg sc q24h  
Enoxaparin 1 mg/kg sc q12h  
Tinzaparin 175 u/kg sc q24h |
| **Therapeutic dose UFH** | UFH sc q12h adjusted to a therapeutic mid-interval aPTT or anti-Xa (0.3-0.7 u/mL) |
Reasons to do thrombophilia testing

• Identify the reason for a thrombotic episode
• Determine the duration of anticoagulation
• Determine the approach to VTE in subsequent pregnancies
• Identify a reason for adverse pregnancy outcomes
# Does genetic thrombophilia cause recurrent pregnancy loss?

<table>
<thead>
<tr>
<th>Thrombophilia</th>
<th>Study #</th>
<th>Patients</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>14</td>
<td>3753</td>
<td>3.04 (2.16-4.3)</td>
</tr>
<tr>
<td>Prothrombin gene mutation</td>
<td>9</td>
<td>2087</td>
<td>2.05 (1.18–3.54)</td>
</tr>
<tr>
<td>MTHFR</td>
<td>8</td>
<td>1818</td>
<td>0.98 (0.55–1.72)</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>8</td>
<td>2026</td>
<td>15.42 (5.90–40.38)</td>
</tr>
<tr>
<td>IgG anticardiolipin antibodies</td>
<td>6</td>
<td>2724</td>
<td>4.68 (2.96–7.40)</td>
</tr>
<tr>
<td>IgM anticardiolipin antibodies</td>
<td>3</td>
<td>1597</td>
<td>4.03 (0.84–19.34)</td>
</tr>
<tr>
<td>β2 Glycoprotein I antibodies</td>
<td>5</td>
<td>1788</td>
<td>2.12 (0.69–6.53)</td>
</tr>
</tbody>
</table>

Opatrný L, et al. J Rheumatol 2006; 33:2214–21
Factor V Leiden is not associated with pregnancy complications

- Prospective multicenter study of 5188 singleton pregnancies (≤14 weeks)
- Exclusions- Known FVL or APS, Previous VTE, pregnancy loss
- Primigravida- 32%
- Lab Testing at 24 weeks
- Conclusion- FVL not associated with pregnancy complications
# Thromboprophylaxis to prevent pregnancy loss

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Intervention</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brenner, 2000</td>
<td>50 pts. w/ thrombophilia RPL</td>
<td>Enox 40 daily</td>
<td>Live birth 20% to 75%</td>
</tr>
<tr>
<td>Brenner, 2003</td>
<td>183 pts.</td>
<td>Enox 40 qd or q12h</td>
<td>Live birth 28% to 79%</td>
</tr>
<tr>
<td>Gris, 2004</td>
<td>160 pts.</td>
<td>Enox 40 vs. ASA 100</td>
<td>Enox 86%, ASA 29%</td>
</tr>
<tr>
<td>Laskin, 2009</td>
<td>88 pts.</td>
<td>Dalte 5000 + ASA 81 vs. ASA 81</td>
<td>D+ASA 77.8%, ASA 79.1%</td>
</tr>
<tr>
<td>Greer, 2010</td>
<td>283 pts.</td>
<td>Enox 40 + ASA 75 Control</td>
<td>Enox+ASA 77.6%, Control 79.3%</td>
</tr>
<tr>
<td>Visser, 2011</td>
<td>207 pts.</td>
<td>Enox 40, Enox 40 +ASA 100, ASA 100</td>
<td>Enox- 71%, Enox +ASA- 65%, ASA – 61%</td>
</tr>
</tbody>
</table>
LMWH does not improve pregnancy outcomes: The ALIFE Study

364 women with at least 2 pregnancy losses

- Placebo (N=121)
  - Completed Study (N=103)

- Aspirin 80 mg (N=120)
  - Completed Study (N=97)

- Aspirin 80 mg + Nadroparin 2850 IU (N=123)
  - Completed Study (N=99)

Kaandorp S et al. NEJM 2010
Table 1. Baseline Characteristics of the Patients.

<table>
<thead>
<tr>
<th></th>
<th>Aspirin plus Nadroparin (N=123)</th>
<th>Aspirin Only (N=120)</th>
<th>Placebo (N=121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean — yr</td>
<td>34±5</td>
<td>33±5</td>
<td>34±5</td>
</tr>
<tr>
<td>≥36 yr — no. (%)</td>
<td>47 (38.2)</td>
<td>41 (34.2)</td>
<td>44 (36.4)</td>
</tr>
<tr>
<td>Body-mass index †</td>
<td>25.4±4.9</td>
<td>25.0±4.8</td>
<td>24.6±4.1</td>
</tr>
<tr>
<td>Daily smoking, ≥1 cigarette — no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily alcohol consumption, ≥8 g — no. (%) ‡</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dutch nationality — no. (%)</td>
<td>102 (82.9)</td>
<td>102 (85.0)</td>
<td>102 (84.3)</td>
</tr>
<tr>
<td>Pregnant at time of randomization — no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscarriage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range) — no.</td>
<td>3 (2–15)</td>
<td>3 (2–9)</td>
<td>3 (2–12)</td>
</tr>
<tr>
<td>≥3 miscarriages — no. (%)</td>
<td>73 (59.3)</td>
<td>71 (59.2)</td>
<td>74 (61.2)</td>
</tr>
<tr>
<td>≥1 late miscarriage — no. (%)</td>
<td>40 (32.5)</td>
<td>38 (31.7)</td>
<td>35 (28.9)</td>
</tr>
<tr>
<td>Previous live birth — no. (%)</td>
<td>53 (43.1)</td>
<td>45 (37.5)</td>
<td>46 (38.0)</td>
</tr>
<tr>
<td>Inherited thrombophilia — no. (%) §</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>105</td>
<td>99</td>
<td>98</td>
</tr>
<tr>
<td>One or more defects</td>
<td>13 (12.4)</td>
<td>17 (17.2)</td>
<td>17 (17.3)</td>
</tr>
<tr>
<td>FactorV Leiden mutation</td>
<td>5 (4.8)</td>
<td>7 (7.1)</td>
<td>9 (9.2)</td>
</tr>
<tr>
<td>Prothrombin G20210A mutation</td>
<td>1 (1.0)</td>
<td>3 (3.0)</td>
<td>0</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>2 (1.9)</td>
<td>1 (1.0)</td>
<td>2 (2.0)</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>4 (3.8)</td>
<td>5 (5.1)</td>
<td>7 (7.1)</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>2 (1.9)</td>
<td>3 (3.0)</td>
<td>0</td>
</tr>
<tr>
<td>Polycystic ovary syndrome — no. (%)</td>
<td>4 (3.3)</td>
<td>3 (2.5)</td>
<td>6 (5.0)</td>
</tr>
</tbody>
</table>

Kaandorp S et al. NEJM 2010
Nadroparin resulted in no increase in live birth rate

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aspirin plus Nadroparin</th>
<th>Aspirin Only</th>
<th>Placebo</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intention-to-treat population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>123</td>
<td>120</td>
<td>121</td>
<td></td>
</tr>
<tr>
<td>Live birth — no. (%)</td>
<td>67 (54.5)</td>
<td>61 (50.8)</td>
<td>69 (57.0)</td>
<td>0.63</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>0.96 (0.76 to 1.19)</td>
<td>0.89 (0.71 to 1.13)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Absolute difference in live-birth rate (95% CI) — %</td>
<td>-2.6 (-15.0 to 9.9)</td>
<td>-6.2 (-18.8 to 6.4)</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Women who became pregnant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of patients</td>
<td>97</td>
<td>99</td>
<td>103</td>
<td></td>
</tr>
<tr>
<td>Live birth — no. (%)</td>
<td>67 (69.1)</td>
<td>61 (61.6)</td>
<td>69 (67.0)</td>
<td>0.52</td>
</tr>
<tr>
<td>Relative risk (95% CI)</td>
<td>1.03 (0.85 to 1.25)</td>
<td>0.92 (0.75 to 1.13)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Absolute difference in live-birth rate (95% CI) — %</td>
<td>2.1 (-10.8 to 15.0)</td>
<td>-5.4 (-18.6 to 7.8)</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

Kaandorp S et al. NEJM 2010
Thrombophilia did not affect live birth rate

Kaandorp SP et al. NEJM 2010
Thrombophilia Testing for RPL

• Antiphospholipid syndrome
  – aPTT
  – dRVVT
  – Anticardiolipin antibodies
  – Beta 2 glycoprotein I antibodies

• Inherited thrombophilia
  – Testing not indicated at present
Thromboprophylaxis in APS for recurrent pregnancy loss

- Antiphospholipid syndrome
  - Dalteparin 5000 units sc q24h + ASA 81 mg
  - Enoxaparin 40 mg sc q24h + ASA 81 mg
  - UFH 5000 sc q12h + ASA 81 mg
Questions ?