THROMBOPHILIA

CME - 1389

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Thrombosis

Coagulation in the wrong place and at the wrong time
A *thrombus* is a blood clot that is formed inside a blood vessel, sometimes blocking blood flow.
Pathophysiology of Thrombosis

1. Abnormalities of blood flow
2. Abnormalities of the vessel wall
3. Abnormalities of blood composition

Rudolf Virchow’s Triad

1821-1902
Pathophysiology of Venous Thrombosis

1. Abnormalities of blood flow:
   Stasis: low – grade activation of coagulation factors and platelets - red clots composed of fibrin and RBC

2. Abnormalities of the vessel wall

3. Abnormalities of blood composition

1821-1902
Rudolf Virchow’s Triad
Pathophysiology of Arterial Thrombosis

1. Abnormalities of blood flow:
turbulence (high shear)

2. Abnormalities of the vessel wall:
   Atherosclerosis (chronic vessel disease); rupture of atheroma:
   platelets deposition; TF exposure
   activation of coagulation factors:
   white clot composed mostly of
   platelets and some fibrin

3. Abnormalities of blood composition

1821-1902
Rudolf Virchow’s Triad
Pathophysiology of Thrombosis

1. Abnormalities of blood flow
2. Abnormalities of the vessel wall
3. Abnormalities of blood composition:
   Hereditary and Acquired Thrombophilias

1821-1902
Rudolf Virchow’s Triad
Thrombotic Disorders

Thrombosis = closure of a vessel

**Arterial**
- Myocardial infarction
- Stroke
- Peripheral art. Dis.

**Venous**
- Deep venous thrombosis (DVT)
- Pulmonary Embolism (PE)

**Thrombus**
- mainly platelets (white thrombus)
- mainly red cells (red thrombus)
Thrombosis

Risk Factors for Thrombosis

Hereditary thrombophilia

Acquired thrombophilia

Atherosclerosis

Surgery trauma

Estrogens

Immobility

Inflammation

Malignancy
Risk Factors for Thromboembolic Disease

- Immobility
- Genetic Factors
- Trauma
- Surgery
- Cancer
- Antiphospholipid Antibodies
- Other Diseases
- Antiphospholipid Abs
- Pregnancy
- Genetic Factors
Thrombophilia = Hypercoagulable state

Thrombophilia = Tendency to Thrombosis

HEREDITARY

ACQUIRED
Thrombosis
Most persons with a thrombophilia do not develop thrombosis.

Thus, thrombophilia must be considered in the context of:

- other risk factors for incident thrombosis, or
- Predictors of recurrent thrombosis, when estimating the need for primary or secondary prophylaxis, respectively.

With rare exceptions, the therapy for acute thrombosis is no different for those with than for those without a recognized thrombophilia.
Hereditary (familial or primary) Thrombophilia

Strongly Supportive Data

- Antithrombin deficiency
- Protein C deficiency
- Protein S deficiency
- Activated protein C resistance
- Factor V Leiden
- Prothrombin G20210A
- Homocystinuria
Hereditary (familial or primary) Thrombophilia -2

**Supportive Data**
- Increased plasma factors I (fibrinogen), II (prothrombin), VIII, IX, XI
- Factor XIII polymorphisms
- Hyperhomocysteinemia
- Dysfibrinogenemia
- Reduced tissue factor pathway inhibitor

**Weakly Supportive Data**
- Reduced protein Z and Z-dependent protease inhibitor
- Tissue plasminogen activator deficiency
- Increased plasminogen activator inhibitor (PAI)-1
- Increased thrombin-activatable fibrinolysis inhibitor
- Hypoplasminogenemia and dysplasminogenemia
- Hypofibrinolysis
Acquired or secondary Thrombophilia

- Strongly Supportive Data

- Active cancer
- Chemotherapy (L-asparaginase, thalidomide, antiangiogenesis therapy)
- Myeloproliferative Neoplasms
- Heparin-induced thrombocytopenia
- Nephrotic syndrome
- Intravascular coagulation and fibrinolysis/DIC
- Thrombotic Thrombocytopenic
- Sickle cell disease
- Oral contraceptives
- Estrogen therapy
- Pregnancy/postpartum state
- Selective estrogen receptor modulator therapy (tamoxifen and raloxifene)
- Antiphospholipid antibodies (lupus anticoagulant, anticardiolipin antibody, anti-β2 glycoprotein-1 antibody)
- Paroxysmal nocturnal hemoglobinuria
- Wegener granulomatosis
Acquired or secondary Thrombophilia-2

- **Supportive Data**
  - Inflammatory bowel disease
  - Thromboangiitis obliterans (Buerger disease)
  - Behçet syndrome
  - Varicose veins
  - Systemic lupus erythematosus
  - Venous vascular anomalies (e.g., Klippel Trenaunay syndrome)
  - Progesterone therapy
  - Infertility “therapy”
  - Hyperhomocysteinemia
  - HIV infection
  - Dehydration
## Epidemiology

### Inherited Thrombophilic Disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Prevalence in Normals (%)</th>
<th>Frequency in Patients with VTE (%)</th>
<th>Relative Risk of First Episode of DVT++</th>
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<tbody>
<tr>
<td>Factor V Leiden (heterozygous)</td>
<td>0.05-4.8*</td>
<td>18.8</td>
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<tr>
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<tr>
<td>Factor V with R2 mutation (heterozygous with FVL)</td>
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<tr>
<td>Protein C deficiency</td>
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<td>Hyperhomocysteinemia**</td>
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<td>Elevated factor IX level</td>
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<td>2.8</td>
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<tr>
<td>Elevated factor XI level</td>
<td>10</td>
<td>19</td>
<td>2.2</td>
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<tr>
<td>Elevated lipoprotein (a) level</td>
<td>7</td>
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<td>3.2</td>
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<tr>
<td>Elevated thrombin-activatable fibrinolysis inhibitor (TAFI)</td>
<td>9</td>
<td>14</td>
<td>1.7</td>
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# Frequency of Inherited Thrombophilias Among Healthy Subjects and Unselected and Selected Patients with Venous Thrombosis

<table>
<thead>
<tr>
<th>Inherited thrombophilia</th>
<th>Healthy subjects</th>
<th>Unselected patients</th>
<th>Selected patients</th>
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<tbody>
<tr>
<td>Protein C deficiency</td>
<td>0.2 – 0.4</td>
<td>3.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>—</td>
<td>2.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>0.02</td>
<td>1.9</td>
<td>4.3</td>
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<tr>
<td>Factor V Leiden</td>
<td>4.8</td>
<td>18.8</td>
<td>40</td>
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<tr>
<td>G20210A prothrombin</td>
<td>2.7</td>
<td>7.1</td>
<td>16</td>
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Thrombophilia: clinical manifestations

- Superficial or deep vein thrombosis (DVT), pulmonary embolism
- Thrombosis of “unusual” venous circulations (e.g., cerebral, hepatic, mesenteric, and renal veins; possibly arm, portal, and ovarian veins; not retinal vein or artery)
- Warfarin-induced skin necrosis
- Possibly arterial thrombosis (e.g., stroke, acute myocardial infarction)
- Recurrent fetal loss
- Purpura fulminans (neonatalis or adult)
- Possibly complications of pregnancy (e.g., intrauterine growth restriction, stillbirth, severe pre-eclampsia, abruptio placentae)
The predominant clinical manifestation of thrombophilia is venous thromboembolism.

Thrombophilia may rarely present as purpura fulminans (e.g., neonatalis or adult) or warfarin-induced skin necrosis.

Most clinical studies have failed to show a consistent association between thrombophilia and myocardial infarction or stroke.

Thrombophilia may also present as recurrent fetal loss and possibly as stillbirth or other complications of pregnancy.
Deep Venous Thrombosis (DVT)

= closure of veins

occurs mostly in leg & thigh veins
Etiologies

- **Most-common thrombophilias**
  - Factor V Leiden
  - Prothrombin G20210A
  - Homocysteinemia (acquired or inherited)

- **Less-common thrombophilias**
  - Increased clotting factors
    - Elevated factor VIII (FVIII) levels are often found in patients with venous thrombosis, but routine testing is controversial
  - Protein C deficiency
  - Protein S deficiency
  - Antithrombin deficiency
  - Impaired clot lysis (dysfibrinogenemia, abnormal fibrinolysis)

- **Antiphospholipid syndrome** is an acquired thrombophilic state
Factor V Leiden

- Genetics and pathophysiology

  - The factor V Leiden (FVL) point mutation is the most common inherited thrombophilia.

  - Single base pair substitution (guanine \( \Rightarrow \) adenine at nucleotide 1691) of factor V gene which prevents cleavage of coagulation factor Va by activated protein C.

  - FV Leiden = Arginine 506 – Glutamine in factor V.
Factor V Leiden

- Accounts for >90% of patients with activated protein C resistance (APC-R)
  - During normal hemostasis, APC limits clot formation by proteolytic inactivation of factors Va and VIIIa
  - **FVL** prevents inactivation of factor Va by APC at the normal rate, increasing the risk for thrombosis

- **Functional tests for APC-R** are generally used as a screening test for FVL
  - *DNA tests are used to confirm positive screening tests and to differentiate between heterozygotes and homozygotes*

- **Autosomal dominant inheritance**
  - Heterozygous carriers have a 5- to 10-fold increased risk
  - Homozygous carriers have a 50- to 100-fold increased risk
Factor V Leiden

- Clinical Presentation

- **Venous thromboembolism (VTE)** is the most common type of thrombotic event
  - Recurrent VTE is generally uncommon in heterozygous patients unless additional risk factors are present
  - Risk of recurrent VTE is increased in homozygous carriers
- Recurrent miscarriage in the second trimester of pregnancy
Factor V Leiden

- Additional risk factors
  - Presence of factor V R2 A4070G mutation in addition to FVL mutation increases risk of thrombotic event 10-fold
  - Many patients with FVL mutation and recurrent episodes of thrombosis have more than one genetic risk factor (e.g., concomitant prothrombin [factor II] G20210A mutation, protein C deficiency, homocystinemia)
  - Acquired factors such as pregnancy, oral contraceptives, hormone replacement therapy, and immobilization increase the risk
Prothrombin G20210A

- Genetics and pathophysiology
  - The prothrombin G20210A mutation is the second most common inherited thrombophilia
    - Results in elevated levels of plasma prothrombin which leads to hypercoagulability (gain of function)
    - Detected using DNA tests
      - Factor II (prothrombin) activity is not an appropriate test
  - Autosomal dominant inheritance
    - A single copy of the G20210A mutation increases the lifetime risk of venous thrombosis by 3-11% while possessing two copies of the mutation leads to even greater risk
Prothrombin G20210A

- Clinical Presentation
  - VTE
  - Pregnancy complications

- Additional risk factors
  - Combined heterozygosity for the prothrombin G20210A mutation and FVL leads to earlier onset, higher rate of recurrence and more severe thrombotic events than either by itself
  - Risk of thrombosis appears increased during pregnancy and with oral contraceptive use
Protein C Deficiency

Pathophysiology

Protein C is a vitamin K-dependent plasma anticoagulant that inactivates factors $\text{Va}$ and $\text{VIIa}$ after being activated to APC by thrombin-thrombomodulin.

- Inherited protein C deficiency is uncommon and may be either quantitative (type I) or qualitative (type II).
  - Autosomal dominant inheritance
  - **Functional assays** (rather than antigenic assays) are preferred for diagnosis
  - Protein C levels vary with age
Protein C Deficiency

Pathophysiology

- **Protein C levels are decreased** in *acute thrombotic states*, *disseminated intravascular coagulation (DIC)*, *liver disease*, *malnutrition* (vitamin K deficiency) and with *warfarin therapy*
  - Elevated FVIII levels (acute phase reactant) may interfere in some functional assays and result in *falsely decreased* values
- **Increased protein C levels** may be seen in *diabetes*, *nephrotic syndrome*, *during pregnancy*, and in patients on *oral contraceptives*
  - **Heparin and direct thrombin inhibitors** may interfere in some functional assays, resulting in *falsely elevated* values
Protein C Deficiency

- Clinical Presentation
  - Additional risk factors likely necessary to provoke thrombosis
  - VTE in heterozygotes
  - Neonatal purpura fulminans (DIC) in homozygous infants
  - Warfarin-induced skin necrosis is seen rarely
Protein S Deficiency

Pathophysiology

- Protein S is a vitamin K-dependent plasma anticoagulant which acts as a cofactor for activated protein C

- Protein S exists in 2 forms
  - Free protein S represents 40% of the total and is physiologically active
  - Bound protein S (attached to C4b-binding protein) represents 60% of the total and possesses no anticoagulant activity
Protein S Deficiency

Pathophysiology, cont

Inherited protein S deficiency is uncommon and may be either quantitative (type 1) or qualitative (type 2)

- Autosomal dominant inheritance
- **Antigenic tests** for free protein S are preferred for diagnosis
- Free protein S values are higher in males than in females
Protein S Deficiency

- Pathophysiology, cont

- Protein S values are decreased in acute thrombotic states, nephrotic syndrome, inflammatory syndromes (due to increased C4b-binding protein), DIC, liver disease, malnutrition (vitamin K deficiency), pregnancy, estrogen therapy, and with warfarin therapy.

  - Elevated FVIII levels may interfere in some functional assays and result in falsely decreased values.

  - APC resistance may interfere in some functional assays and result in falsely decreased values.

  - Heparin and direct thrombin inhibitors may interfere in some functional assays and result in falsely elevated values.
Protein S Deficiency

- Clinical Presentation
  - Additional risk factors likely necessary to provoke thrombosis
  - VTE most common, arterial thrombosis may occur
  - Neonatal purpura fulminans (DIC) in homozygous infants
  - Warfarin-induced skin necrosis is seen rarely
Antithrombin Deficiency

- **Pathophysiology**
  - Antithrombin (AT) is a *plasma anticoagulant* that inactivates thrombin, factor Xa and other activated clotting factors
    - Antithrombin activity is enhanced by heparin-like glycosaminoglycans on the endothelial surface and by pharmaceutical heparin
  - Inherited antithrombin deficiency may be either quantitative (*type 1*) or qualitative (*type 2*)
    - Autosomal dominant inheritance
    - *Functional assays are preferred for diagnosis*
  - **Decreased** antithrombin occurs in acute thrombotic states, liver disease, DIC, nephrotic syndrome and heparin therapy; mild decreases may be seen in pregnancy or with oral contraceptive use.
  - **Increased** AT may occur with long-term warfarin therapy in some patients
Antithrombin Deficiency

- Clinical Presentation
  - VTE
  - Recurrent thrombosis may occur even in the absence of additional risk factors
  - Some deficient patients are resistant to heparin therapy
## The original thrombophilia trio: partial deficiencies of regulators of thrombin generation

<table>
<thead>
<tr>
<th></th>
<th>Antithrombin</th>
<th>protein C</th>
<th>protein S</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inheritance:</strong></td>
<td>autosomal dominant, incomplete penetrance</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prevalence:</strong></td>
<td>1:600&lt;sup&gt;1&lt;/sup&gt; - 5000&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1: 200&lt;sup&gt;3&lt;/sup&gt;-600&lt;sup&gt;4&lt;/sup&gt;</td>
<td>1: 800-4000&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>VTE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prevalence:</strong></td>
<td>1-3%</td>
<td>1-3%</td>
<td>1-3%</td>
</tr>
<tr>
<td><strong>Relative risk?</strong></td>
<td>~100</td>
<td>~10</td>
<td>~10</td>
</tr>
</tbody>
</table>

<sup>1</sup> AT activity  <sup>2</sup> AT antigen  <sup>3</sup> PC antigen  <sup>4</sup> PC activity  <sup>5</sup> fPS and total PS
Hyperhomocysteinemia

- Independent risk factor for thromboembolic events
- **Most** patients with hyperhomocysteinemia do not have genetic mutations or polymorphisms
- Regardless of the underlying etiology, hyperhomocysteinemia is the result of deranged homocysteine metabolism which may be **acquired** (deficiency of vitamins B6, B12, or folic acid) or **inherited** (deficiency of cystathionine β-synthase or expression of a thermolabile form of methylenetetrahydrofolate reductase)
Hyperhomocysteinemia-2

- Thrombotic risk is most closely associated with increased fasting plasma homocysteine levels regardless of the underlying etiology
  - Plasma homocysteine testing is recommended rather than DNA-based tests
Procoagulant effect of homocysteine on vascular endothelium

- Prolonged exposure of endothelial cells to homocysteine impairs EDRF
- Homocysteine stimulated the proliferation of smooth muscle cells – atherogenesis
- Homocysteine decreases thrombomodulin expression and activity – decreased activation of protein C
- Homocysteine inhibits the ATIII binding activity of endothelial heparan sulfate proteoglycan
- Homocysteine inhibits ADP-ase activity of HUVEC
- Homocysteine inhibits binding of tPA – decreased fibrinolysis
- Homocysteine induces TF activity
Methylenetetrahydrofolate reductase (MTHFR) mutations

- **Genetics**
  - Autosomal recessive inheritance
  - The **most common** genetic defects of homocysteine metabolism are the MTHFR mutations C677T and A1298C
    - The C677T mutation results in a **thermolabile variant** of MTHFR

- **Clinical Presentation**
  - Elevated plasma homocysteine levels have been associated with **atherosclerotic disease, VTE** and **arterial thrombosis**
Currently recommended indications for thrombophilia testing include:

- Idiopathic or recurrent venous thromboembolism;
- A first episode of venous thromboembolism at a “young” age (e.g., < 40 years);
- A family history of venous thromboembolism (in particular, a first-degree relative with thrombosis at a young age);
- Venous thrombosis in an unusual vascular territory (e.g., cerebral, hepatic, mesenteric, or renal vein thrombosis); and
- Neonatal purpura fulminans or Warfarin-induced skin necrosis.
- Population screening is not recommended
Diagnosis

□ Indications for testing for inherited disorders

□ Situations where testing should be considered

■ Idiopathic thrombosis in patient $\leq 50$ years of age
■ Recurrent thrombosis
■ Unusual sites of thrombosis
■ First-degree relatives with thromboses
■ Thrombotic event during pregnancy
■ Thrombotic event while taking oral contraceptives
Diagnostic Thrombophilia Testing: What Should I Test For?

- A **complete history** and **physical examination** is **mandatory** when evaluating individuals with a recent or remote history of thrombosis, with special attention given to patient age at onset, location of prior thromboses, and results of objective diagnostic studies documenting thrombotic episodes.
Thrombophilia: How Do You Decide Who to Test?
<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Arterial</th>
<th>Venous</th>
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</thead>
<tbody>
<tr>
<td>Factor V Leiden</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Antithrombin deficiency</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Protein C deficiency</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Protein S deficiency</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Hyperhomocysteinemia</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Lupus Anticoagulant</td>
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Stratification of Potentially Thombophilic Patients

<table>
<thead>
<tr>
<th>Clinical History</th>
<th>“Weekly”</th>
<th>“Strongly”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of onset &lt; 50</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Recurrent thrombosis</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Positive family history</td>
<td>-</td>
<td>+</td>
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</table>
Screening Evaluation
For “Strongly Thrombophilic” Patients

- Test for Factor V Leiden
- Genetic test for prothrombin gene mutation 20210A
- Functional assay of antithrombin
- Functional assay of protein C
- Functional assay of protein S
- Clotting test for lupus anticoagulant/ELISA for cardiolipin antibodies
- Measurement of fasting total plasma homocysteine
Screening Laboratory Evaluation
For “Weekly Thrombophilic” Patients

- Test for Factor V Leiden
- Genetic test for prothrombin gene mutation G20210A
- Measurement of fasting total plasma homocysteine
- Clotting assay for lupus anticoagulant/ELISA for cardiolipin antibodies
Diagnosis – Lab Tests Request

- If testing indicated, consider the following
  - Activated protein C resistance (with or without reflex to FVL mutation); Factor V R2 A4070G mutation
  - Prothrombin mutation
  - Antithrombin activity
  - Protein C activity
  - Free protein S
  - Factor VIII activity (testing other factor activities such as FVIII and FIX is controversial and not currently recommended)
  - Testing for less common disorders is available if results are uninformative and additional testing is indicated
Hereditary hypercoagulability factors tested by ARMS - PCR

<table>
<thead>
<tr>
<th>Factors</th>
<th>Samples (n)</th>
<th>Wild-type homozygous</th>
<th>Risk allele heterozygous</th>
<th>Risk allele homozygous</th>
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<td>102</td>
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<td>Factor V (1691G/A)</td>
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<td>Factor V HR2 (4070A/G)</td>
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<td>Factor VII (Gln353Arg)</td>
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<td>E4/E4</td>
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Reference DNA samples were tested for hereditary hypercoagulability by ARMS. The number of samples tested per allelic constellation are indicated.
Diagnosis – Lab Tests

- Indications for testing for inherited disorders
  - Many tests are **altered** by **acute thrombotic states** (acute phase response, consumption of factors and anticoagulant factors) and **anticoagulant therapies**
    - Delay testing **2-3 months** after acute event
      - Preferable to discontinue oral anticoagulant therapy at least **2 weeks to 1 month** before testing
    - **Heparin** and **direct thrombin inhibitors** interfere with many of the tests and should be discontinued prior to testing
      - Heparin interference in tests may be due to therapy with unfractionated or low molecular weight heparin or heparin contamination from a line draw
    - **DNA-based tests are not affected by an acute phase response or anticoagulant therapy**
Diagnosis – Lab Tests

- Indications for testing for inherited disorders
  - Consider repeating abnormal functional or antigenic testing before making a definitive diagnosis of an inherited thrombophilia
    - Low results can be obtained due to patient condition/biologic variability, medications, and assay variability or interference
    - Consider patient age and gender when interpreting results; normal ranges vary by age and gender
Differential Diagnosis

- **Provoked/acquired** causes of thrombophilia are **more common** than hereditary causes and should be considered when evaluating patients with thrombosis.

- Examples of provoked/acquired causes of thrombophilia include:
  - Antiphospholipid antibodies/lupus anticoagulant
  - Malignancy
  - Long distance travel
  - Trauma
  - Surgery
  - Immobilization
  - Presence of a central venous catheter
  - Pregnancy/postpartum
Antiphospholipid Syndrome, APS

- Most common acquired thrombophilia
- Described by Hughes (1983)

A syndrome characterized by the association of:

- thrombosis, obstetric complications and/or thrombocytopenia
- antibodies against phospholipids or against proteins bound to phospholipids.
Antiphospholipid Syndrome - Etiology

- Combination of genetic background and environmental factors: infection, trauma, drugs
  - infections – molecular mimicry with B2GPI

<table>
<thead>
<tr>
<th>Infectious agent</th>
<th>Presence of anticardiolipin antibodies</th>
<th>APS manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C virus</td>
<td>IgG</td>
<td>Thrombosis, brain infarction</td>
</tr>
<tr>
<td>Varicella-zoster virus</td>
<td>IgG, IgM</td>
<td>Pulmonary embolism, thrombosis</td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td>IgG</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>IgG, IgM</td>
<td>Thrombosis</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>IgG, IgM, IgA</td>
<td>Leg ulcer necrosis, arterial and venous thrombosis and embolism, vasculitis, livedo reticularis, thrombocytopenia</td>
</tr>
</tbody>
</table>
Antiphospholipid Antibodies

10% of healthy donors, 30-50% of SLE patients

- **LA antibodies** are directed against *plasma proteins* bound to anionic phospholipids

- **aCL antibodies** are directed against *phospholipids* bound to proteins
  - Can be IgA, M, or G (subclasses 1-4)
  - IgG (esp G2) associated with a greater risk of APS

- **Anti β_2GPI antibodies** are directed against a *plasma protein* that binds phospholipid with high affinity
Antiphospholipid Antibodies

- **Lupus Anticoagulant (LA) Antibodies**
  - Prolonged coagulation in phospholipid-dependent in vitro tests (aPTT, PT, dRVVT)
  - Failure to correct with 50:50 mix
  - Correction of coagulation time by adding phospholipid

- **Anticardiolipin (aCL) Antibodies**
  - ELISA assay in the presence of bovine B2GPI

- **Anti-Beta 2 Glycoprotein I Antibodies (β₂GPI)**
  - ELISA assay using human B2GPI coated plates
  - most specific
Beta 2 Glycoprotein I

- natural inhibitor of coagulation and platelet aggregation
  - Inhibits contact activation of coagulation cascade
  - Inhibits conversion of prothrombin to thrombin
- most aPL antibodies recognize Domain I of $\beta_2$GPI
- binding of antibody increases binding affinity for phospholipids
**APS Pathophysiology**

- **αPL**
  - **platelets**
    - Activate platelet aggregation
  - **Coagulation cascade**
    - Inhibit Protein C, Protein S, thrombomodulin, antithrombin III, fibrinolysis
  - **Endothelial cells**
    - ↑ TF, adhesion molecules, proinflammatory cytokines
  - **Placental tissue**
    - ↓ Trophoblastic cell growth, ↑ apoptosis, ↓ IL-3
  - **Complement system**
APS Pathophysiology

Binding of APA to endothelial cells and platelets

Platelet activation and aggregation

Endothelial cell activation

Proinflammatory cytokines

T-cell immune response

Coagulation

Putative “second” hit
- trauma
- infection
- nonimmune procoagulant factors

Anti-lamin B1 antibody exerts protective effect

APA binding to β₂-glycoprotein, prothrombin, proteins C and S, and annexin V interferes with coagulation cascade

- Protein C activation
- Antithrombin III activity
- Annexin V binding
- Fibrinolysis
- Tissue factor activity

ANTIPHOSPHOLIPID SYNDROME
Diagnosis - Clinical Criteria

- **Vascular thrombosis**: arterial, venous, or small vessel, in any tissue or organ, confirmed by objective validated criteria.

- **Pregnancy morbidity**:  
  - Unexplained fetal death at or beyond 10 weeks gestation.  
  - Premature birth before 34 weeks gestation because of eclampsia, severe pre-eclampsia, or placental insufficiency.  
  - Three or more consecutive spontaneous abortions before 10 weeks gestation.
Diagnosis - Laboratory criteria

- **Lupus anticoagulant**, present on at least 2 occasions, at least 12 weeks apart
- **Anticardiolipin antibodies** (ACA), IgG or IgM > 30 units for both, present on at least 2 occasions, at least 12 weeks apart
- **Anti-beta-2-glycoprotein I antibodies** (anti-B2GPI), IgG or IgM > 20 units for both, present on at least 2 occasions, at least 12 wks apart

A diagnosis of APS should not be made if a period of greater than five years separates the clinical event and positive laboratory test.
Acquired Thrombophilia

Lupus Anticoagulant (LA)

Antibodies against phospholipid

Inhibit phospholipid-dependent pathways (prolonged APTT that is not corrected by normal plasma)

Causes arterial & venous thrombosis

Mechanism of thrombosis unknown

Clinical associations: SLE, thrombocytopenia, fetal loss, miscarriages
Lupus Anticoagulant (LA)

Inhibits the APTT by preventing the intrinsic pathway factors, IXa and VIII, from successfully attaching to phospholipid surfaces and activating factor X

Prolonged APTT, yet liability for thrombosis rather than bleeding
Euro-Phospholipid Study

- 1000 patients with APS: 820 (82%)F, 180 (18%)M, F:M (5:1)
- Mean age at the onset 34 ± 13 years
- 53% primary APS,
  - 36% APS associated to SLE,
    - 5% associated to lupus-like syndrome,
    - 5% associated to other diseases.
- Catastrophic APS - 8 (0.8%) patients, in 6 at the onset.
<table>
<thead>
<tr>
<th>Manifestations</th>
<th>No.</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peripheral thrombosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep vein thrombosis</td>
<td>389</td>
<td>(38.9)</td>
</tr>
<tr>
<td>Superficial thrombophlebitis in legs</td>
<td>117</td>
<td>(11.7)</td>
</tr>
<tr>
<td>Arterial thrombosis in legs</td>
<td>43</td>
<td>(4.3 )</td>
</tr>
<tr>
<td>Venous thrombosis in arms</td>
<td>34</td>
<td>(3.4)</td>
</tr>
<tr>
<td>Arterial thrombosis in arms</td>
<td>27</td>
<td>(2.7)</td>
</tr>
<tr>
<td><strong>Neurologic manifestations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Migraine</td>
<td>202</td>
<td>(20.2)</td>
</tr>
<tr>
<td>Stroke</td>
<td>198</td>
<td>(19.8)</td>
</tr>
<tr>
<td>Transient ischemic attack</td>
<td>111</td>
<td>(11.1)</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>70</td>
<td>(7   )</td>
</tr>
<tr>
<td>Multiinfarct dementia</td>
<td>25</td>
<td>(2.5)</td>
</tr>
<tr>
<td>Chorea</td>
<td>13</td>
<td>(1.3)</td>
</tr>
<tr>
<td><strong>Pulmonary manifestations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>141</td>
<td>(14.1)</td>
</tr>
<tr>
<td>Pulmonary hypertension</td>
<td>22</td>
<td>(2.2)</td>
</tr>
<tr>
<td><strong>Cardiac manifestations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valve thickening/dysfunction</td>
<td>116</td>
<td>(11.6)</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>55</td>
<td>(5.5)</td>
</tr>
<tr>
<td>Angina</td>
<td>27</td>
<td>(2.7)</td>
</tr>
<tr>
<td>Myocardiopathy</td>
<td>29</td>
<td>(2.9)</td>
</tr>
<tr>
<td>Vegetations</td>
<td>27</td>
<td>(2.7)</td>
</tr>
<tr>
<td><strong>Intraabdominal manifestations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal manifestations</td>
<td>27</td>
<td>(2.7)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>15</td>
<td>(1.5)</td>
</tr>
<tr>
<td>Splenic infarction</td>
<td>11</td>
<td>(1.1)</td>
</tr>
<tr>
<td>Pancreatic infarction</td>
<td>5</td>
<td>(.5 )</td>
</tr>
<tr>
<td>Addison's syndrome</td>
<td>4</td>
<td>(.4 )</td>
</tr>
<tr>
<td>Hepatic manifestations</td>
<td>7</td>
<td>(7   )</td>
</tr>
<tr>
<td><strong>Cutaneous manifestations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Livedo reticularis</td>
<td>241</td>
<td>(24.1)</td>
</tr>
<tr>
<td>Ulcers</td>
<td>55</td>
<td>(5.5)</td>
</tr>
<tr>
<td>Pseudovasculitic lesions</td>
<td>39</td>
<td>(3.9)</td>
</tr>
<tr>
<td>Digital gangrene</td>
<td>33</td>
<td>(3.3)</td>
</tr>
</tbody>
</table>
**Euro-Phospholipid Study - clinical features in 1000 patients with APS**

**Ophthalmologic manifestations**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amaurosis fugax</td>
<td>54</td>
<td>5.4%</td>
</tr>
<tr>
<td>Retinal artery thrombosis</td>
<td>15</td>
<td>1.5%</td>
</tr>
<tr>
<td>Retinal vein thrombosis</td>
<td>9</td>
<td>.9%</td>
</tr>
<tr>
<td>Optic neuropathy</td>
<td>10</td>
<td>1%</td>
</tr>
</tbody>
</table>

**Hematological manifestations**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thrombocytopenia (&lt; 100,000/μl)</td>
<td>296</td>
<td>29.6%</td>
</tr>
<tr>
<td>Hemolytic anemia</td>
<td>97</td>
<td>9.7%</td>
</tr>
</tbody>
</table>

**Obstetric manifestations (pregnant female = 590)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-eclampsia</td>
<td>56</td>
<td>9.5%</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>26</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

**Fetal manifestations (pregnancies = 1580)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early fetal losses (&lt; 10 weeks)</td>
<td>560</td>
<td>35.4%</td>
</tr>
<tr>
<td>Late fetal losses (&gt; 10 weeks)</td>
<td>267</td>
<td>16.9%</td>
</tr>
</tbody>
</table>
Catastrophic APS

**Preliminary criteria:**

1. Involvement of three or more organs or tissues
2. Development of manifestations simultaneously or in < 1 week
3. Histopathologic evidence of small-vessel occlusion in at least one type of tissue
4. Presence of lupus anticoagulant, anticardiolipin antibodies or both

**Definite diagnosis:**

All four criteria met

**Probable diagnosis:**

2 organs or tissues involved, and the 2nd, 3rd and 4th criteria met; or

All 4 criteria met and negative test for LA or anticardiolipin antibody > 6 wks after the first positive test or death within that period; or
First, 2nd and 4th criteria met; or
First, 3rd and 4th criteria met and development of a third manifestation in >1 wk but <1 mo despite anticoagulation

APS – under-recognized autoimmune disease that accounts for a significant proportion of thromboembolic disease and recurrent pregnancy loss

The etiology and pathophysiology involves aPL as “first hit” and environmental factors, including infection as “secondary hit”

APS - complex disorder with evolving diagnostic criteria

Anticoagulation rather than immunosuppression is the current mainstay of therapy

Well-designed prospective studies are required to complete the understanding of the optimal treatment.
Multifactorial Pathophysiology of Thrombosis in Cancer Patients

1. Abnormalities of blood flow:
   a) Immobilization and bed rest
   b) Vascular compression from bulky tumor
   d) Hyperviscosity

2. Abnormalities of the vessel wall:
   a) Direct vascular invasion by tumor
   b) Reduction in PA within vascular endothelium

3. Abnormalities of blood composition:
   a) Elevated I, V, VII, VIII factors
   b) Decreased clearance of the activated factors
   c) Activation of coagulation system by the tumor cells

1821-1902 Rudolf Virchow’s Triad
VTE incidence increases with age

Anderson Arch Int Med 1991, 151: 933-938

Fig 1.—Incidence rate of clinically recognized deep vein thrombosis and/or pulmonary embolism per 100 000 population. The increase in rates for both male and female patients is well approximated by an exponential function of age. The modeled rate for male patients (upper curve) is significantly higher ($P<.05$) than that for female patients (lower curve).
PCR-RFLP for detection
Hereditary Thrombophilia

Prothrombin G20210A

F-VL

MTHFR
Reverse Dot Blot - PCR

7. Homozygous MTHFR-A1298C
Homzygous F V-Leiden

6. Heterozygous MTHFR-A1298C
7. Homozygous MTHFR-A1298C
Thank you,
Any question?