Thrombosis: What’s the Risk?
Will I be one of the UNFORTUNATE ones?

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Diagnostica Stago
Thrombosis: What’s the Risk?

Objectives

- List genetic factors potentially involved in thrombosis, including Protein C, Protein S, Antithrombin and Factor V Leiden.
- Discuss Lupus Anticoagulants and their role in thrombosis.
- Describe situational risk in thrombosis, such as malignancies, air travel and high-risk surgeries.
- Evaluate approaches to thrombosis testing: whom and what do we test?
- Summarize thrombosis risk in case studies and how it relates to the diagnostic team.
March is Blood Clot Awareness Month!
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BLOOD CLOT AWARENESS MONTH 2019: “TOGETHER WE CAN INCREASE AWARENESS AND SAVE LIVES”

March is Blood Clot Awareness Month (BCAM), which is perhaps one of the most important times of the year for the National Blood Clot Alliance, because it is so important, in so many ways, to the patients, caregivers, healthcare professionals, and advocates we serve. Not only does BCAM provide an opportunity to unite as a community to share resources and stories, it also provides a crucial opportunity

Stop the Clot’s Instagram Awareness Campaign

Overview of Venous Thromboembolism (VTE)
Venous Thromboembolism (VTE)

- VTE affects 300,000 to 600,000 Americans annually, results in ~100,000 deaths\(^1\)
- ~30% of patients presenting with suspected VTE have PE, with 20 - 25% presenting as sudden death, diagnosed at autopsy (27,000 people)\(^1\)
- PE is the leading cause of preventable hospital death and maternal mortality in the US\(^1\)
- Treat with anticoagulation for customized length depending on patient needs, family history, comorbidities, bleeding risk, other medications, etc.\(^1\)
- Inherited VTE risk higher in Northern European populations, but acquired risk similar in all ethnic groups evaluated\(^2\)

Monetary Cost of VTE

- Two thirds of cases occur in outpatients
- Diagnostics and prescription costs between $7,594 - $16,644 per patient
- Contributes well over $2 billion in total cost to the healthcare system annually

Risk Factors for DVT and PE

**HYPERCOAGULABLE STATE**
- Malignancy
- Pregnancy and peri-partum period
- Oestrogen therapy
- Trauma or surgery of lower extremity, hip, abdomen or pelvis
- Inflammatory bowel disease
- Nephrotic syndrome
- Sepsis
- Thrombophilia

**VASCULAR WALL INJURY**
- Trauma or surgery
- Venepuncture
- Chemical irritation
- Heart valve disease or replacement
- Atherosclerosis
- Indwelling catheters

**CIRCULATORY STASIS**
- Atrial fibrillation
- Left ventricular dysfunction
- Immobility or paralysis
- Venous insufficiency or varicose veins
- Venous obstruction from tumour, obesity or pregnancy

**Virchow’s Triad**
- Stasis of blood flow
- Endothelial injury
- Hypercoagulability

accessed Mar 5, 2019
Due to difference in clinician practices, there is a much higher number of patients suspected of PE in the US and prescribed imaging to confirm PE compared to outside US suggesting the higher rates of unnecessary imaging procedures in the US compared to Europe and Canada.
Signs and Symptoms are Nonspecific

- VTE is one disease entity with two patterns of clinical presentation:
  - Deep Vein Thrombosis (DVT) is blood clot in leg veins
  - Pulmonary Embolism (PE) is a clot in lungs which migrated from leg veins

- DVT
  - Pain tenderness and/or swelling in the calf or leg
  - Discoloration of the calf that can extend to the foot
  - Symptoms of PE

- PE
  - Difficulty breathing
  - Sharp chest pain worsened by taking a deep breath
  - Blood in the sputum
  - Rapid heart rate

[Source: www.emedicinehealth.com/slideshow_pictures_deep_vein_thrombosis_dvt/article_em.htm; accessed Mar 5, 2019]
Anatomy of a Clot

http://www.rxlist.com/blood_clots/page8.htm; accessed Mar 5, 2019

Post Thrombotic Syndrome

Genetic Risk

- Protein C
- Protein S
- Antithrombin
- Factor V Leiden
Thrombophilia

- Defined as a tendency toward thrombosis, mainly venous thromboembolism (VTE).
- Weakens the ability to cope with environmental challenges such as immobilization.
- Not in itself the cause of thrombosis but better described as a risk factor.
- May be inherited or acquired
  - Inherited thrombophilia is a genetically determined tendency to develop thrombosis.
  - Acquired thrombophilia results from a number of conditions including advancing age and antiphospholipid antibody syndrome.

Thrombophilia – VTE Risk Factor Model

VTE Risk Factor Model

**Intrinsic Thrombosis Risk**

- **Genes**
  - Anticoagulant deficiencies
  - Antithrombin 20-fold \(\uparrow\) RR
  - Protein S 10-fold \(\uparrow\)
  - Protein C 10-fold \(\uparrow\)
  - Prothrombin 3-fold \(\uparrow\)
  - Factor V Leiden 3-8 fold \(\uparrow\)

- **Acquired Risk Factors**
  - Age
  - Previous VTE
  - Cancer
  - Obesity

**Triggering Factors**

- Estrogens
- Pregnancy
- Surgery
- Immobilization

**Prophylaxis**

- **Thrombosis Threshold**

**VTE**

*from* Folsom A
Hereditary Thrombophilia

- AT was the first identified hereditary thrombophilia followed by PC and PS.
- Mutations of the F5 (FV Leiden) and F2 (Prothrombin) genes were subsequently identified.
- Only these 5 have been unequivocally associated with venous thrombosis i.e. at least 2 fold increased risk.
- AT, PC and PS deficiencies are “high risk” thrombophilia compared to “low risk” F5 and F2 mutations.
- Clinical studies have shown these conditions are associated with an increased risk of first VTE but not with an increased risk of recurrence.
Coagulation/Hemostasis is a balance between pro- & anti-coagulant mechanisms → bleed & clot, → hemorrhage & thrombosis

- Triggering agents
- pro-enzyme → enzyme (serine-protease: FIIa, FVIIa, FIXa, FXa)
- Cofactors (FVa & FVIIIa)

- Serine-protease inhibitor: Antithrombin (AT)
- Cofactors/inhibitors: Protein C / S
- Tissue factor pathway inhibitor (TFPI)

Fibrinogen → Fibrin
Antithrombin (AT) - Basics

- Glycoprotein, liver synthesis
  - Major inhibitor of factors IIa and Xa
  - Normal plasma range ~ 80 - 120 %
  - Plasma concentration: 2.5 µM (0.15 g/l)

- AT congenital deficiency : usually 40 - 60 %

- Physiological variations

- Acquired AT deficiency (i.e. heparin resistance)
Antithrombin mode of action

- Endothelial GAG (HS) (= natural Heparin)
- Combines with antithrombin
- Acts Locally on the endothelial surface
AT Function

AT + heparin + factor IIa

\[ \text{AT-heparin-IIa reversible complex} \]

\[ \text{[ AT-IIa ] + heparin irreversible complex} \]

*in which factor IIa is inactive*
Hereditary AT Deficiency

- Deficiency causes reduced inhibition of FXa and thrombin leading to increased thrombin generation.

- **Type I: True deficiency**
  - Quantitative defect characterized by a parallel reduction in antigen and activity (both activity & antigen ↓)

- **Type II: Qualitative defects** characterized by a reduction in activity levels relative to antigen levels (activity ↓; antigen N)
  - Subtypes: reactive site defect, heparin-binding site defect, pleiotropic defect.

- Both are predominantly associated with VTE early in life.
- Type I incidence is 0.20:1000 versus 1.45:1000 Type II
Antithrombin – Physiological Variation and Deficiency

**Physiological variation**
- AT level is low until the age of 6 months
- Marked decrease after the 13th week of pregnancy and during post partum
- AT level in women up to the menopause is lower than in men

**Acquired AT deficiency**
- Heparin therapy +++,
- Severe liver disease
- Nephrotic syndrome
- Oral contraceptives
- Preeclampsia
- DIC
PC and PS Function

Protein C System Overview

Protein C (PC) - Basics

- Synthesised by the liver
  - Vitamin-K dependent glycoprotein
  - Normal plasma range: 70 - 130 %
  - Levels for heterozygous PC deficient patients between 25 % and 70 %
  - Prevalence of the congenital deficiency in the general population: 0.1 - 0.3 %

- Physiological variations
  - At birth, low PC levels are observed due to liver immaturity
  - PC increases slightly during pregnancy and during the puerperium
  - In adults, the protein C level is independent of age and sex

- Acquired deficiencies
  - Oral Anticoagulant therapy, liver disorders, nephrotic syndrome, DIC, etc.
PC Deficiency - Hereditary

- **Type I:**
  - True congenital deficiency
  - Reduced PC antigen and PC activity

- **Type II:**
  - Abnormal molecule
  - Normal PC antigen, but reduced PC activity
PC is activated by thrombin to APC which has anticoagulant, anti-inflammatory and cytoprotective functions.

Deficiency causes impaired inactivation of FVa and FVIIIa leading to increased thrombin generation.

Type I: Quantitative defect characterized by a parallel reduction in antigen and activity (to about 50% of normal in heterozygote's).

Type II: Qualitative defects characterized by a reduction in activity levels relative to antigen levels.

The prevalence of deficiency about 1 in 300.
Protein S (PS) - Basics

- **Produced in the liver**
  - Vitamin-K dependent glycoprotein
  - Normal range (free PS Ag)
    - Men: 108% (SD 16.5%)
    - Women: 88% (SD 19.5%)

- **2 forms of protein S:**
  - Free PS (40 %), cofactor for activated PC
  - PS bound to a regulator of the complement system, the C4b Binding Protein, functionally inactive

- **C4bBP is a large multimeric protein** (octopus-shaped, its shorter arm binding PS)
Hereditary PS Deficiency

- PS is a cofactor for inactivation of FVa and FVIIIa by APC
- 60% of PS is bound to C4b-binding protein; 40% is free and active.
- Type I: Quantitative defect characterized by a parallel reduction in antigen and activity
- Type II: Qualitative defects characterized by a reduction in activity levels relative to free and total antigen levels (very rare).
- Type III: Reduced activity and free antigen with normal total antigen
- The prevalence of deficiency is about 1:1000
Protein S (PS) – Physiological Variation and Deficiency

**Physiological variation**
- At birth, low total PS, normal free PS level
- Total and free PS levels are lower in woman than in man, and increase with age
- PS levels decrease during pregnancy

**Acquired deficiency**
- Warfarin, DIC, liver disease, oral contraception, nephrotic syndrome, AIDS, inflammatory syndrome
Two forms of Protein S

60% (reversible)

40% (active form for hemostasis)

aPC: Activated PC; PS: Protein S; C4bBP: C4b Binding-Protein
C4b Binding Protein (C4b-BP) - Basics

- Protein involved in the complement system
  - MW = 570KD
  - High affinity for Protein S (Quasi-totality bounded to PS in the plasma)
Main role of PS in PC system


aPC: Activated PC; TM:Thrombomodulin; PS: Protein S; C4bBP: C4b Binding-Protein; EPCR: Endothelial Cell PC Receptor; FIIa : thrombin ; PC : Protein C ; FVa & FVIIa : Factors V & VIII activated; FVai & FVIIIai : Factors Va & VIIIa inactivated
### AT, PC & PS Deficiencies

**Table 1** Abnormalities caused by loss-of-function of anticoagulant proteins

<table>
<thead>
<tr>
<th>Deficiency</th>
<th>Antigen level$^*$</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antithrombin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>Low</td>
<td>Low (with or without heparin)</td>
</tr>
<tr>
<td>Type II (reactive-site defect)</td>
<td>Normal</td>
<td>Low (with or without heparin)</td>
</tr>
<tr>
<td>Type II (heparin-binding-site defect)</td>
<td>Normal</td>
<td>Low with heparin, or normal with no heparin added $^4$</td>
</tr>
<tr>
<td>Type II (pleiotropic effects)</td>
<td>Decreased</td>
<td>Low (with or without heparin)</td>
</tr>
<tr>
<td><strong>Protein C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Type II</td>
<td>Normal</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Protein S</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>Low total and free antigen</td>
<td>Low</td>
</tr>
<tr>
<td>Type II</td>
<td>Normal total and free antigen</td>
<td>Low</td>
</tr>
<tr>
<td>Type III</td>
<td>Normal total and low free antigen</td>
<td>Low</td>
</tr>
</tbody>
</table>

$^*$ In general, heterozygote carriers show “low” (~50% of the normal) or “decreased” (50–70% of the normal) antigen and/or activity levels, and homozygote carriers nearly undetectable levels. To discriminate between different types of deficiencies, protein activity is tested first and, if low, antigen level is then tested. $^4$ Laboratory testing for type II antithrombin deficiency is made with and without added heparin, to discriminate reactive-site (low antithrombin activity in plasma in both instances) from heparin-binding site defect (low activity only with heparin added).
# Pediatric Reference Ranges – AT, PC, PS

## Component

<table>
<thead>
<tr>
<th>Component</th>
<th>Neonatal Levels vs. Adult</th>
<th>Effect on Hemostasis</th>
</tr>
</thead>
</table>
| Coagulation Factors| ↓ Prothrombin, FVII, FIX, FX, FXI, FXII  
↔ Fibrinogen, FV  
↓ Fibrinogen function  
↔ or ↑ FVIII | Reduced prothrombotic capacity               |
| Primary Hemostasis | ↑ vWF  
↓ Platelet function  
↔ Platelet level | Increased capacity of primary hemostasis   |
| Fibrinolysis       | ↓ Plasminogen, tPA, α2-antiplasmin  
↔ or ↑ PAI | Decreased fibrinolytic capacity             |
| Coagulation inhibitors | ↓ AT, PC, PS (total), TFPI (free)  
↑ PS (free) | Reduced antithrombotic capacity             |

### Abbreviations

- **F**: factor
- **vWF**: von Willebrand Factor
- **tPA**: tissue plasminogen activator
- **PAI**: plasminogen activator inhibitor
- **AT**: antithrombin
- **PC, PS**: protein C, protein S

### Symbols

- **↑ Higher**
- **↔ Same**
- **↓ Lower**

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In a large study from China of 3,493 adults (1,734 men, 1,759 women; ages 17-83 years), higher age-adjusted PC and PS activities were found in men than women, but no gender-based differences for AT levels.

Factor V Leiden

- Single mutation in Factor V
- Substitution of arginine by glutamine at position 506
- Dysfunctional Factor V
- “Activated Protein C Resistance”

90% of APC resistance due to $FV_{Leiden}$

Heterozygotes – 8-fold increase relative risk (RR)

Homozygotes – 80-fold increase RR
Inherited Risk Factors

Prothrombin Gene Mutation
- Mutation in the untranslated portion of the protein
- Elevated plasma levels of prothrombin, ~115%-130%
- ~1% in Caucasians, low in African, Asian & Native Americans
- ~3-fold increase in relative risk

Hyperhomocysteinemia
- >100 umol/L
- Mutation in enzymes responsible for homocysteine metabolism
- Associated with premature atherosclerosis & arterial thrombotic disease

Lab testing – fasting specimen
- HPLC, enzyme immunoassays, fluorescence polarization immunoassays
- Genetic testing
Other Risks - Elevated FVIII

- Independent risk factor for VTED
  - 100-125%, ~2-fold increase
  - 125-150%, ~3-fold increase
  - >150%, ~5-fold increase
Multi ‘Hit’ Effect

- Same mutation effects different families differently
- Combination of inherited risk factors
  - More severe thrombosis
  - Earlier age

<table>
<thead>
<tr>
<th></th>
<th>One Hit</th>
<th>One Hit</th>
<th>Two Hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% $FV_{Leiden}$</td>
<td>20%</td>
<td>54%</td>
<td>92%</td>
</tr>
<tr>
<td>13% $FV_{Leiden}$</td>
<td>13%</td>
<td>31%</td>
<td>73%</td>
</tr>
<tr>
<td>19% $FV_{Leiden}$</td>
<td>19%</td>
<td>19%</td>
<td>72%</td>
</tr>
</tbody>
</table>
Lupus Anticoagulant Diagnostic Guidelines
LA Guidelines - Chronology


• **2014** Clinical and Laboratory Standardization Institute (CLSI) *Laboratory Testing for the Lupus Anticoagulant, H60-A, April 2014*.
LA Diagnostic Flow Chart (2009 ISTH Guidelines)

**If family history for aPS OR abnormal screen test**

- **PT/aPTT**
  - NORMAL → STOP
  - ABNORMAL → RUN TT

- **RUN TT**
  - NORMAL
  - ABNORMAL → STOP

- **DRVV Screen**
  - NORMAL → STOP
  - ABNORMAL (add mixing step)

- **DRVV Confirm**
  - ABNORMAL (add mixing step)

- **PTT LA**
  - NORMAL
  - ABNORMAL (add mixing step)

**Calculate normalized ratio**

**Calculate delta**

**It may be necessary for the lab to rule out other coagulopathies that could coexist**
LA Testing Guidelines – ISTH SSC 2009

**Step 1 – Screen (low PL reagent)**
- LA sensitive aPTT reagent
- dRVVT screen reagent
- Thrombin Time – eliminate prolonged clotting times due to anticoagulation

**Step 2 – Mixing studies**
- Repeat screening tests using a patient 1:1 mix
- 1:1 mix = 1 part patient + 1 part pool normal plasma

**Step 3 – Confirm (high PL reagent)**
- Hexagonal phase PL reagent (Staclot® LA)
- dRVVT confirm

Tests must be repeated > 12 weeks after initial testing; need to demonstrate persistence
Other Helpful Tests to Consider

- Full laboratory aPS profile should include:
  - LA testing (clotting based)
  - Anti β2-GPI ELISA (aβ2-GPI) IgG, IgM
  - Anticardiolipin (aCL) IgG, IgM

- Presence of medium-high titers of aCL and aβ2-GPI of same isotype (i.e. IgG) is in agreement with positive LA and IDs patients with high thrombotic risk

- Thrombin time can help to rule out heparin and other anticoagulant contamination
Triple Positive aPS Patients

- Lupus anticoagulants
- Anti-β2GPI
- Anticardiolipin

## Persistence of Testing Results

<table>
<thead>
<tr>
<th>Test</th>
<th>% positive on repeat testing</th>
<th>Mean follow up time</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>39 of 51 patients (77%)</td>
<td>2.4 years</td>
</tr>
<tr>
<td>Anticardiolipin antibody (moderate – high titer)</td>
<td>65 of 86 patients (75%)</td>
<td>3.5 years</td>
</tr>
<tr>
<td>Anti-β2GPI antibody (moderate – high titer)</td>
<td>11 of 15 patients (76%)</td>
<td>1.0 year</td>
</tr>
</tbody>
</table>

- Repeat aPS results remain stable for at least ¾ of patients regardless of laboratory performing test
- Variation not correlated with aspirin, warfarin, or hydroxyquinoline use
- Indefinite anticoagulation is indicated for most aPS patients

LA is the primary predictor of adverse pregnancy outcome after 12 weeks’ gestation in aPL-associated pregnancies. Anticardiolipin antibody and anti-2GPI, if LA is not also present, do not predict adverse pregnancy outcome.

## Comparison of LA Guidelines

### Preanalytical conditions

<table>
<thead>
<tr>
<th>Area of recommendation</th>
<th>ISTH 2009</th>
<th>BCSH 2012</th>
<th>CLSI 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample preparation</td>
<td>Double centrifugation</td>
<td>Double centrifugation</td>
<td>Double centrifugation</td>
</tr>
<tr>
<td>Assays to use</td>
<td>DRVVT and aPTT</td>
<td>DRVVT plus aPTT or others</td>
<td>DRVVT and aPTT and/or others</td>
</tr>
<tr>
<td>Testing order</td>
<td>Screen-mix-confirm</td>
<td>Screen-mix-confirm</td>
<td>Screen-confirm-mix</td>
</tr>
<tr>
<td>Ratio derivation</td>
<td>NPP denominator</td>
<td>NPP denominator</td>
<td>RI mean denominator</td>
</tr>
<tr>
<td>RI/cutoffs</td>
<td>99th percentile</td>
<td>97.5th percentile (if Gaussian)</td>
<td>97.5th percentile (if Gaussian)</td>
</tr>
</tbody>
</table>

### Choice of tests & Methodology

<table>
<thead>
<tr>
<th>Calculations for phospholipid-dependence</th>
<th>ISTH 2009</th>
<th>BCSH 2012</th>
<th>CLSI 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>% correction of screen by confirm, or LA ratio (screen/confirm)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mixing test</th>
<th>ISTH 2009</th>
<th>BCSH 2012</th>
<th>CLSI 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perform on 1:1 mixture with NPP; Interpret with ICA or mixing test-specific cutoff</td>
<td>Perform on 1:1 mixture with NPP</td>
<td>Perform on 1:1 mixture with NPP; Interpret with ICA or mixing test-specific cutoff</td>
<td></td>
</tr>
</tbody>
</table>

### Results Interpretation

<table>
<thead>
<tr>
<th>Testing patients on VKAs</th>
<th>ISTH 2009</th>
<th>BCSH 2012</th>
<th>CLSI 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undiluted plasma if INR &lt; 1.5; Mix with NPP if INR &gt; 1.5 &lt; 3.0</td>
<td>Screen and confirm on 1:1 mixture with NPP; TSVT + ET or PNP</td>
<td>Screen and confirm on 1:1 mixture with NPP; TSVT + ET or PNP</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Testing patients on UFH</th>
<th>ISTH 2009</th>
<th>BCSH 2012</th>
<th>CLSI 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interpret with caution</td>
<td>Not recommended</td>
<td>Not recommended</td>
<td>Can detect LA in some cases where heparin neutralizer is effective</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interpretive reporting</th>
<th>ISTH 2009</th>
<th>BCSH 2012</th>
<th>CLSI 2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recommended</td>
<td>Recommended</td>
<td>Recommended</td>
<td>Recommended</td>
</tr>
</tbody>
</table>

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Antiphospholipid Syndrome (aPS) Overview
Clinical Manifestations of aPS

- Transient ischemic attacks
- Stroke
- Pulmonary embolism
- Vegetations
- Myocardial infarction
- Valve thickening/dysfunction
- Pre-eclampsia/eclampsia
- Live birth with prematurity
- Livedo reticularis
- Skin ulcers
- Thrombocytopenia
- Early pregnancy loss
- Live birth with intrauterine growth restriction
- Late pregnancy loss
- Deep vein thrombosis
- Inferior extremity superficial thromboflebitis
Antiphospholipid Syndrome (aPS) Diagnosis

Thrombosis and/or Recurrent pregnancy loss

Persistently positive test for LAC and/or aCL and/or anti-β2GPI

Diagnosis

Consequences for treatment & prognosis

Antiphospholipid Syndrome (aPS) is an auto-immune condition characterized by a hypercoagulable state:

- Blood clots in arteries and veins
- Pregnancy complications such as recurrent miscarriages or severe preeclampsia

Rare syndrome, more prevalent in women than in men.

Primary aPS: absence of any other related disease

Secondary aPS: with other auto-immune disease such as Lupus erythematosus (SLE)

In rare cases, aPS can lead to rapid organ failure due to generalized thrombosis = Catastrophic antiphospholipid syndrome

Treatment:
Anticoagulation (UFH) to reduce thrombosis risk & improve pregnancy prognosis (no VKA / teratogenic)
Antiphospholipid syndrome (aPS) is caused by antiphospholipid antibodies (APL):
- Heterogeneous group of antibodies
- Able to bind phospholipids (PL) to prolong PL-dependent tests

In vivo APL cause Thrombosis: Modulate TF expression, enhance binding to platelets, and interfere with antithrombotic mechanisms

In vitro APL cause prolongation of clotting time: Normally thrombosis state = time shortening. In vitro artifact due to test principle: in clotting time factors are activated by PL in the assay. If PL are monopolized by APL, factor activation is decreased (prolongs clotting time).
aPTT Reagent – PL Concentration

Routine aPTT
50%
PL

Sensitive aPTT
25%
PL

Confirmatory
100%
PL

× = PL particles
LA Effect on Clotting Tests

**Patient without LA**

- FX → FXa → FXa → FXa → FXa → Clotting Time Normal

**Patient with LA**

- FX → FXa → FXa → FXa → FXa → Lupus → Clotting Time Prolonged
Phospholipid Dependency of Protein C Pathway

Prothrombin \( \xrightarrow{\text{PL}} \) IIa

\( \xrightarrow{\text{PL}} \) fPS

APC

VIIIa or VIIIa

\( \xrightarrow{\text{PL}} \) TM

Protein C

PC

TM

FXa/FVa/Ca^{++}

Indicates any phospholipid-dependent activation step
Lupus Anticoagulant Laboratory Tests
LA Testing: Preanalytics

- Double centrifugation (stay away from the platelet layer)

- Platelet Poor Plasma (PPP); < 5000 platelets/µL

- No hemolysis or traumatic draw, discard 1st tube

- Pooled normal plasma (PNP) should be from multiple donors, well characterized for all coagulation factors and platelet poor

- -70° C preferred for freezing; CLSI recommends no more than two weeks at -20° C (H21-A5, H57-A)
Coagulation Assay Mechanisms

**aPTT Based**

**dRVVT Based**
Activated Partial Thromboplastin Time (aPTT)

- Involves activation of FXII by PL and CaCl$_2$
- Reagent composition
  - PL
  - Activator
  - Tests for factors VIII, IX, IX and XII
- Clinical uses for aPTT
  - Factor deficiencies
  - Heparin therapy
  - Circulating anticoagulants
  - Disseminated intravascular coagulation (DIC)
- Potential interference from anticoagulant drugs
  - Warfarin
  - Rivaroxaban, edoxaban (not sensitive to apixaban)
  - Argatroban, dabigatran
dRVVT Screen & Confirm – Principle

**Screening Tests**
- **DRVV Screen**
  - Patient without LA – Low [PL] test

**Confirmation Tests**
- **DRVV Confirm**
  - Patient without LA – High [PL] test

**Ratio < 1.2**

Clotting Time Normal

FX

FXa

FXa

FXa

FXa

FXa

FXa

FXa

FXa

FXa

FXa

FXa

FXa
dRVVT Screen & Confirm – Principle

Screening Tests
DRVV Screen
Patient with LA – Low [PL] test

Confirmation Tests
DRVV Confirm
Patient with LA – High [PL] test

Ratio > 1.2
Effect of LA (+) Sample on Screening aPTT

In the cuvette, LA overwhelms the PL in the aPTT reagent

Reduced concentration of PL results in prolonged clotting times

Clotting times (example):
- Normal plasma: 28.0 – 36.0 seconds
- LA positive: 55 seconds
Effect of LA (+) on aPTT and dRVVT Screen

- In the cuvette, LA overwhelms the PL in the aPTT/dRVV reagent
- Reduced concentration of PL results in prolonged clotting times
- Clotting times (example):
  - PTT-LA
    - Normal: 34.3 – 40.4 seconds
    - LA positive: 62.0 secs.
  - dRVVT screen
    - Normal: 36.8 – 42.8
    - LA positive: 58.0 secs

aPTT/dRVVT screen

25%

PL

= PL particles

= LA
Mixing Studies

- Use in order to rule out factor deficiencies which may prolong clotting times (CT)
- Perform as a 1:1 or 50/50 mix of PNP and patient
- Compare immediate mix to 60 min preincubated mix

Calculate Index of circulating anticoagulant (ICA):

\[
\frac{(CT_{\text{mixture}} - CT_{\text{PNP}}) \times 100}{CT_{\text{patient}}}
\]

Results
1. Correct
2. Fail to Correct
Mixing Study Interpretation
*APTT normal range = 25-35 sec*

<table>
<thead>
<tr>
<th>Sample:</th>
<th>Immediate</th>
<th>60 min</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>46</td>
<td>48</td>
<td>Complete Correction</td>
</tr>
<tr>
<td>PNP</td>
<td>31</td>
<td>32</td>
<td>C/W Factor Deficiency</td>
</tr>
<tr>
<td>1:1 Mix 1</td>
<td>33</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Patient 2</td>
<td>46</td>
<td>48</td>
<td>Incomplete Correction</td>
</tr>
<tr>
<td>PNP</td>
<td>31</td>
<td>32</td>
<td>C/W Inhibitor</td>
</tr>
<tr>
<td>1:1 Mix 2</td>
<td>36</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Patient 3</td>
<td>46</td>
<td>60</td>
<td>Incomplete Correction</td>
</tr>
<tr>
<td>PNP</td>
<td>31</td>
<td>32</td>
<td>Prolongation at 60 min</td>
</tr>
<tr>
<td>1:1 Mix 3</td>
<td>35</td>
<td>48</td>
<td>C/W time dependent inhibitor (Factor VIII Inhibitor)</td>
</tr>
</tbody>
</table>
LA Effect on dRVVT Confirm

- In the cuvette, LA over­whelms the PL in the aPTT/dRVV reagent
- Reduced concentration of PL results in prolonged clotting times
- Clotting times (example):
  - dRVVT confirm
    - Normal: Ratio <1.2
    - LA positive: 1.8
  - Staclot® LA
    - Normal: Δ <8.0 secs
    - LA positive: Δ 25 secs

100%

PL particles

LA
Staclot® LA – Integrated Test System

Steps 1 and 2

<table>
<thead>
<tr>
<th>Patient Buffer</th>
<th>Patient Hex Phos</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNP</td>
<td>PNP</td>
</tr>
<tr>
<td>aPTT-LS</td>
<td>aPTT-LS</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>CaCl₂</td>
</tr>
</tbody>
</table>

Low PL concentration
Tube #1

High PL concentration
Tube #2

T1-T2 = Δ Time
Staclot LA - Principle

**Tube 1**
Without hexagonal PL
- Patient without Lupus
  - FX → FXa
  - Clotting Time Normal

**Tube 2**
With hexagonal PL
- Patient without Lupus
  - FX → FXa → Specific Hexagonal PL neutralizing LA
  - Clotting Time Normal

**CT1 – CT2 < 8 sec**
Staclot LA - Principle

Tube 1
Without hexagonal PL
Patient with LA

Tube 2
With hexagonal PL
Patient with LA

CT1 – CT2 > 8 sec

Clotting Time Prolonged

Specific Hexagonal PL neutralizing LA
Influence of Anticoagulant Drugs on LA Testing

<table>
<thead>
<tr>
<th></th>
<th>Prevalence of abnormal results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dabigatran (110 mg bid)</td>
</tr>
<tr>
<td>PT activity &lt;70%</td>
<td>95</td>
</tr>
<tr>
<td>TT ratio &gt;1.2</td>
<td>100</td>
</tr>
<tr>
<td>aPTT &gt; 40 sec</td>
<td>100</td>
</tr>
<tr>
<td>aPTT &gt; 40 sec, ICA &gt; 10%</td>
<td>100</td>
</tr>
<tr>
<td>dRVVT screen &gt;40 sec</td>
<td>100</td>
</tr>
<tr>
<td>dRVVT &gt;40 sec, ICA &gt;13%</td>
<td>100</td>
</tr>
<tr>
<td>dRVVT screen/confirm NR &gt;1.17</td>
<td>81.8</td>
</tr>
</tbody>
</table>

Lupus Anticoagulant Testing - Case Studies
Case Study # 1

- 11 year old female presents with epistaxis and fever, along with malaise and anorexia. She had an unremarkable physical history with a tonsillectomy at age 9 without excessive bleeding, no relatives with bleeding histories, but taking aspirin for fever.

- Physical exam showed she was afebrile and well nourished but cervical lymphadenopathy present.

# Case Study # 1 Laboratory Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>PT</td>
<td>13.8 sec</td>
<td>11.3 – 14.6 sec</td>
</tr>
<tr>
<td>aPTT</td>
<td>55.0 sec</td>
<td>25 – 34 sec</td>
</tr>
<tr>
<td>aPTT 1:1 mix</td>
<td>43.0 sec</td>
<td>Correction to 25 – 34 sec</td>
</tr>
<tr>
<td>PTT-LA</td>
<td>108.0 sec</td>
<td>36 – 50.1 sec</td>
</tr>
<tr>
<td>dRVVT Screen</td>
<td>43.1 sec</td>
<td>29.6 – 42.9 sec</td>
</tr>
<tr>
<td>Prothrombin</td>
<td>82%</td>
<td>50 – 150%</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>135%</td>
<td>60 – 160%</td>
</tr>
<tr>
<td>Staclot® LA</td>
<td>20.4 sec</td>
<td>Negative &lt; 8 sec</td>
</tr>
</tbody>
</table>

Case Study # 1 – Diagnosis

- Probability of hemophilia A (FVIII deficiency) and hemophilia B (FIX deficiency) are low, along with factor inhibitors.

- LA is most likely present; often are transient in children and associated with viral infections

- LA not expected to cause thrombosis in this case

- Cervical lymphadenopathy presence suggests infectious mononucleosis

Case Study # 2

- 43 year old male presents with an ischemic stroke.

- History of hypertension, but no surgical history available.

- Unremarkable family history, the only medication being taken is a multivitamin.

### Case Study # 2 Laboratory Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelet count</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>PT</td>
<td>12.5 sec</td>
<td>11.3 – 14.6 sec</td>
</tr>
<tr>
<td>aPTT</td>
<td>78.0 sec</td>
<td>25 – 34 sec</td>
</tr>
<tr>
<td>PTT-LA</td>
<td>90.0 sec</td>
<td>36.1 – 50.1 sec</td>
</tr>
<tr>
<td>Thrombin time</td>
<td>16.0 sec</td>
<td>&lt; 18.0 sec</td>
</tr>
<tr>
<td>dRVVT screen</td>
<td>78.6 sec</td>
<td>29.6 – 42.9 sec</td>
</tr>
<tr>
<td>dRVVT Mix</td>
<td>56.0 sec</td>
<td>Correction to 29.6 – 42.9 sec</td>
</tr>
<tr>
<td>dRVVT Confirm</td>
<td>37.5 sec</td>
<td>N/A</td>
</tr>
<tr>
<td>dRVVT Ratio</td>
<td>2.1</td>
<td>&lt; 1.3</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>200</td>
<td>50 – 186%</td>
</tr>
<tr>
<td>Factor VIII inhibit</td>
<td>N/A</td>
<td>Undetectable</td>
</tr>
</tbody>
</table>

Case # 2 Diagnosis and Therapy

- LA is likely

- Due to stroke incidence along with presence of LA, diagnose as positive aPS

- LAs are found in 18% of stroke patients < 44 years old

- Provide LMWH or warfarin therapy

- aPTT cannot be used to monitor heparin therapy; will need to use anti-Xa

LA Testing Conclusions

- Nomenclature for LA can be confusing

- Tests for aPS
  - Clotting studies to detect LA
  - Confirm tests should be based on same format as screen
  - Direct detection of the antibodies using ELISA

- Diagnosis of aPS requires the presence of at least one of the clinical entities: thrombosis, pregnancy morbidity; and at least one positive test

- The positive findings must be persistent when retesting at > 12 weeks
Situational Risk
Risk Factors for DVT and PE

**Virchow’s Triad**

- Hypercoagulability
- Endothelial injury
- Stasis of blood flow

**Hypercoagulable State**
- Malignancy
- Pregnancy and peri-partum period
- Oestrogen therapy
- Trauma or surgery of lower extremity, hip, abdomen or pelvis
- Inflammatory bowel disease
- Nephrotic syndrome
- Sepsis
- Thrombophilia

**Vascular Wall Injury**
- Trauma or surgery
- Venepuncture
- Chemical irritation
- Heart valve disease or replacement
- Atherosclerosis
- Indwelling catheters

**Circulatory Stasis**
- Atrial fibrillation
- Left ventricular dysfunction
- Immobility or paralysis
- Venous insufficiency or varicose veins
- Venous obstruction from tumour, obesity or pregnancy

Malignancies

Why are they a risk?

Solid phase tumors have highest risk

Very high risk: Pancreas & Stomach
High risk: Lung, lymphoma, Gynecologic, Bladder, testicular
Moderate risk: Brain, Kidney Lung

Clinical risk depends on several factors

- Patient related (age, comorbidities, race, gender)
- Cancer related
- Treatment-risk related

Khorana, Alex, Cancer and Coagulation; Am. J. Hematology 87:S82-87, 2012
Malignancies
Why are they a risk?

Therapeutic interventions
- Cancer patients undergoing surgery have a 2x risk of VTE compared with non-cancer patients
- Risk is increased for up to 7 weeks.

Chemotherapy
- Chemotherapy increases risk 2 – 6x that of general population

<table>
<thead>
<tr>
<th>TABLE II. Predictive Model for Chemotherapy-Associated VTE [14]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient characteristics</td>
</tr>
<tr>
<td>Site of cancer</td>
</tr>
<tr>
<td>Very high risk (stomach, pancreas)</td>
</tr>
<tr>
<td>High risk (lung, lymphoma, gynecologic, bladder, testicular)</td>
</tr>
<tr>
<td>Prechemotherapy platelet count 350,000/mm³ or more</td>
</tr>
<tr>
<td>Hemoglobin level &lt;10 g dL⁻¹ or use of red cell growth factors</td>
</tr>
<tr>
<td>Prechemotherapy leukocyte count &gt;11,000/mm³</td>
</tr>
<tr>
<td>Body mass index 35 kg m⁻² or more</td>
</tr>
</tbody>
</table>

High-risk score ≥ 3; intermediate risk score = 1–2; Low-risk score = 0.

Khorana, Alex, Cancer and Coagulation; Am. J. Hematology 87:S82-87, 2012
What about time of day? 
More events happen in the morning.....

Morning Hypercoagulability and Hypofibrinolysis

Kapiotis, Circulation; Vol 96(1):19-21; July 1997
Conditions specific for women

Pregnancy, peri-partum, oral contraceptives, hormone therapy

- Pregnancy
- Peri-partum
- Oral contraceptives
- Hormone therapy
VTE is a leading cause of morbidity and mortality in pregnancy

Venous thrombosis complicates approximately **1.2 in 1,000** deliveries.

Incidence of VTE is similar in antepartum and postpartum periods, but **postpartum period** shorter so higher daily VTE risk.

Increased risk persists until 12 weeks postpartum, with **greatest risk in first 6 weeks** after delivery.

Diagnosis, prevention, and treatment of VTE in pregnancy must consider both **fetal and maternal well-being**.

ASH Clinical Practice Guidelines
Antepartum and Postpartum

What is the patient’s VTE history?

For women not already receiving long-term anticoagulant therapy who have a history of VTE, the panel makes the following recommendations:

<table>
<thead>
<tr>
<th>Prior VTE History</th>
<th>Antepartum Prophylaxis</th>
<th>Postpartum Prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unprovoked VTE (strong recommendation, low certainty)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Provoked VTE, Hormonal risk factor</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Provoked VTE, Non-Hormonal risk factor</td>
<td>No**</td>
<td>Yes</td>
</tr>
</tbody>
</table>

These recommendations were made based on a VTE risk threshold of 2% antepartum and 1% postpartum for recommending LMWH prophylaxis (as long as no current additional risk factors for VTE)
Oral Contraceptives & Menopausal Hormone Therapy

What’s the risk?

- Oral contraceptives (OCs) are considered as one of the most common risk factor of venous thromboembolism (VTE) in childbearing age.
  - Recent research indicate that the odds of VTE may be even higher with newer generations of OCs.
  - The use of second- and third-generation OCs increased the risk of VTE by up to threefold and 4.3-fold, respectively.

- One of rare but severe complications of Menopausal Hormone Therapy (MHT) is venous thromboembolism (VTE).
  - The incidence of VTE rises in parallel to women’s age & body weight.
  - Condition is also linked to hereditary & acquired risk factors.
  - Oral estrogens increase the risk to varying extents; studies have not found an association of increased risk and transdermal treatments.
Surgery
It can be a VTE Risk

- 23 million people have surgery per year
  - Up to 20% of high risk surgical patients acquire DVT, and only 5% are at low risk of VTE
- The most common types of surgery associated with VTE are orthopedic surgeries, especially knee and hip replacements.

- Additional Risk:
  - major surgery
  - injuries that cause vein trauma, like fractures, muscle damage, long-bone breaks, and spinal cord injuries

- In one year, a single 400 bed hospital will have 200 hospital acquired VTEs with 50% of those having been preventable (!!!)
  - Beginning in 2015, the perioperative DVT/PE rate is one of the measures used for Medicare’s Hospital Value-Based Purchasing that links quality to payment
  - Anticoagulation Safety is part of The Joint Commission National Patient Safety Goals

The Joint Commission National Patient Safety Goals (effective January 1st, 2019)
Venous thromboembolism (deep vein thrombosis or pulmonary embolism) can occur as a result of long periods of immobility associated with any form of travel.

The risk of venous thromboembolism (VTE) for most travelers is low.
- For a flight > 4 hours, in healthy individuals, the risk is estimated to be 1 in 6,000. The risk increases with longer duration of travel and with multiple flights within a short period. The risk of pulmonary embolism is much lower.

Some travelers are at increased risk
- older travelers, pregnant women, those with a previous history of VTE or recent surgery, those with certain blood clotting disorders, malignancy, certain heart conditions and those taking estrogen containing medicines

To reduce the risk of VTE, travelers should regularly move their legs (walk when possible or flex and extend the ankles to encourage blood flow from the lower legs).

Those at increased risk of VTE should seek advice from their health care provider and consider the use of properly fitted compression socks. Low molecular weight heparin therapy may also be recommended for certain higher risk patients.

https://travelhealthpro.org.uk/factsheet/54/venous-thromboembolism
Additional risk factors
Yes, there are more……

✦ Obesity
  - people with obesity have 2 times the risk of VTE as people with normal weight, and the higher the weight, the higher the risk.

✦ Immobility
  - Prolonged immobility combined with other major risk factors increases the likelihood of VTE
  - Physical inactivity

✦ Age
  - Patients older than 40 years are at higher risk, and that risk doubles with each subsequent decade

✦ Prior risk
  - Patients with a previous episode of VTE have a high chance of recurrence.

✦ Family history of VTE
  - especially if this is in a first-degree relative (parent, sibling, child)

AHA:https://www.heart.org/en/health-topics/venous-thromboembolism/risk-factors-for-venous-thromboembolism-vte
**Genetic & Acquired “Hits”**

- **Oral Contraceptive (OC) - independent risk factor for VTED**
  - ~4-9-fold increased risk for VTED

- **OC use & FV Leiden**
  - ~32-fold increased risk for heterozygotes
  - ~320-fold increased risk for homozygotes

- **OC use & II gene mutation**
  - ~16-fold increased risk for heterozygotes

- **OC use, II gene mutation & FV Leiden**
  - ~150-fold increase in risk!
Thrombophilia Testing Recommendations

- Protein C
- Protein S
- Antithrombin
- Factor V Leiden
Hereditary Thrombophilia Testing

- Thinking has moved from more liberal to more conservative for many reasons, including over diagnosis due to inappropriate testing, ill affects of over aggressive management, and cost.
- Testing for hereditary defects should only be done in cases where knowing the result would alter patient management.

Stephan Moll, MD, HTRS Workshop, Apr 2016
Whom to Evaluate

- Prevalence does not justify general population screening
- Lab workup is $$$$$
- Patients with history of unexplained thromboembolism
  - First degree family members
Hereditary Thrombophilia Testing

- **ACCP Therapy for VTE Disease (2012)**
  - No clear recommendation for testing

- **NICE VTE Management (2012)**
  - Do not offer thrombophilia testing to patients who are continuing anticoagulation
  - Do not offer thrombophilia testing to patients with provoked VTE
  - Consider testing for heritable thrombophilia for patients with unprovoked VTE if there is a 1st degree relative with VTE and it is planned to stop anticoagulation

- **ASH Choosing Wisely recommendations (2013)**
  - Do not test for thrombophilia in adult patients with VTE occurring in the setting of major transient risk factors (surgery, trauma, prolonged immobility)
Risk of First VT: Testing may be useful in certain situations e.g. in families with AT, PC, PS, or homozygous FVL, but limited to women who intend to become pregnant or who would like to use oral contraceptives.

Risk of Recurrent VT: Observational studies show that patients who have had VTE and have hereditary thrombophilia, are at most at a slightly increased risk for recurrence therefore testing is not recommended.

Risk of Pregnancy Complications: The data are unclear as to whether intervention on the basis of thrombophilia testing is beneficial.

Guidelines and recommendations point to minimal testing for hereditary thrombophilia.

Acquired Deficiencies

- Acquired deficiencies of AT, PC and PS are much more common than hereditary deficiencies.
- All 3 may be decreased in liver disease, thrombosis, surgery, DIC and L-asparaginase treatment.
- All 3 are low from birth to 6 months and PC may be low through adolescence.
- Pregnancy and estrogen use decrease PS and AT to a lesser extent.
- Anticoagulation affects levels
  - Heparin decreases AT
  - Coumadin decreases PS and PC
- All of these effects need to be ruled out before diagnosing hereditary deficiency.
Dx of Congenital Deficiencies

Important to rule out acquired deficiencies!

- OAC therapy lowers PC, PS
- Heparin therapy lowers AT
- Pregnancy & OC use lowers PS
- Acute thrombosis – elevated FVIII, fibrinogen
  - Interference of functional PS, PC assays
- Severe liver disease – lowers coagulation proteins
- Young age – low levels of AT, PS, PC
American College of Medical Genetics Consensus Statement:

- Age <50, any venous thrombosis
- Unusual sites
- Recurrent venous thrombosis
- Venous thrombosis with strong family history
- Venous thrombosis in pregnant woman or OC users
- Family members of young patients with venous thrombosis
- Myocardial infarction in female smokers under 50
Recommendations for $\text{FV}_{\text{Leiden}}$

American College of Medical Genetics Consensus Statement

- Positive by functional, then DNA test to confirm
- Heparin or known LA, omit functional (unless using modified assay)
- Relatives of know $\text{FV}_{\text{Leiden}}$ – omit functional
Testing Strategy – AT, PC, PS

♦ **Functional first:**
  - Detects all types of deficiencies (either reduced or dysfunctional protein).
  - Exception – subset of PC deficiency not detected using a chromogenic test system. Use clot-based instead.

♦ **Antigenic assays:**
  - Used to characterize deficiencies.
Classifying Genetic Abnormalities

In general:
- Type I – decreased level of functional protein
- Type II – Normal levels but dysfunctional (loss of function)

Exception - Protein S:
- Type I – low activity & low free & total PS
- Type II – low activity, normal free & total
- Type III – low activity, low free, normal total
<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Limitations &amp; Interferences</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT - functional</td>
<td>Chromogenic</td>
<td>Heparin &gt;1 IU/mL will falsely elevate functional PC, PS</td>
</tr>
<tr>
<td>AT - antigenic</td>
<td>Immunologic</td>
<td>OAC decreases PC, PS</td>
</tr>
<tr>
<td>PC - functional</td>
<td>Clotting</td>
<td>FVIII &gt;250% falsely lowers functional PC, PS assays</td>
</tr>
<tr>
<td>PC - antigenic</td>
<td>ELISA</td>
<td>Circulating inhibitors may falsely raise functional PC, PS</td>
</tr>
<tr>
<td>PS - functional</td>
<td>Clotting</td>
<td></td>
</tr>
<tr>
<td>PS - antigenic</td>
<td>Immunologic, ELISA</td>
<td></td>
</tr>
</tbody>
</table>
Laboratory Testing - APCr

**APC Resistance**
- APTT-based assay, +/-APC
- Result-ratio between clotting time in the presence and absence of APC
- Normal ~ 2-5 (instrument dependent)

**Limitations**
- Baseline APTT must be normal
- Antiphospholipid antibodies may yield low ratio (false positive)
- APTT reagents display varying sensitivity to APC
Genetic Testing

- **DNA based method – FV\textsubscript{Leiden} & II Gene**
  - PCR amplification of the defective region
  - Restriction enzyme cleavage
  - Agarose gel electrophoresis
    - Compare number & size of fragments

- **Limitations**
  - technical expertise required
  - Lengthy procedure
  - Contamination
  - Results may have implications for family members, insurance discrimination????
Activated Protein C Deficiency Testing Algorithm

AT Deficiency Testing Algorithm

- Is the functional antithrombin level low?
  - NO: No antithrombin deficiency
  - YES: Was there recent or is there current full-dose heparin therapy?
    - NO: Is there evidence of an acquired antithrombin deficiency as found in cases with recent thrombosis or surgery, DIC, liver dysfunction, estrogen therapy, pregnancy, fatty liver of pregnancy, proteinuria, or L-asparaginase therapy?
      - NO: Perform antithrombin antigen measurement
        - Is the antigen level low?
          - YES: Antithrombin deficiency type I*: low antigen level and low functional level (quantitative deficiency)
          - NO: Antithrombin deficiency type II*: normal antigen level and low functional level (quantitative deficiency)
        - Repeat testing at a later date to confirm the deficiency, unless the diagnosis is already established in a relative
    - YES: Repeat the functional antithrombin level when the patient has not received full-dose heparin therapy for at least 10 days
    - Repeat antithrombin level when these conditions are not present, if possible, because all can decrease antithrombin.** If not possible, consider testing first-degree relatives.

PC Deficiency Testing Algorithm

PS Deficiency Testing Algorithm

AT, PC & PS Diagnostic Algorithm

AT Assay Limitations

- **Thrombin-based** antithrombin assay results might be falsely elevated by endogenous **heparin-cofactor II**: bovine thrombin resists heparin-cofactor II and may be preferable.
- **Direct thrombin inhibitors** (e.g., argatroban etc) falsely increase results with thrombin—but not Xa-based assays (102% → 135% with 2 ug/mL argatroban (unpublished observations))
- **Xa-based** antithrombin assay results might **overestimate** in some families for unclear reasons.

Elizabeth Van Cott MD, THSNA Meeting 2012

bovine thrombin based activity assays are a good option for AT activity testing
PC Assay Limitations

An APTT clotting-based assay is a good choice for PC testing when used correctly because it detects Type II deficiencies.


Some clot-based assays might also miss the rare type II variant Asn2Ile (131C>T) in the Gla region:

- Antigen 96%
- Chromogenic (3 assays) 98%
- Staclot (PTT-based) 50%
- Cryocheck Clot C (RVV-based) 69.5%
- HemosIL Proclot C (PTT-based) 91%

Reduced binding to phospholipid and EPCR

Cooper PC et al, Int J Lab Hematol 2011; 33:451-456
An aPTT clotting-based assay is a good choice when used correctly because it detects Type II deficiencies and may be less sensitive to FVL.


Comparison of PS Assays – Mulder et al

- 2 Total PS, 4 Free PS and 3 PS Activity assays were compared
- Within run = 10 replicates on one day
- Between run = duplicates run on 5 consecutive days

<table>
<thead>
<tr>
<th>Test</th>
<th>Normal</th>
<th>Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CVintra</td>
<td>CVinter</td>
</tr>
<tr>
<td>TPS</td>
<td>1.9</td>
<td>4.9</td>
</tr>
<tr>
<td>PA</td>
<td>4.4</td>
<td>8.2</td>
</tr>
<tr>
<td>FPS</td>
<td>3.1</td>
<td>1.9</td>
</tr>
<tr>
<td>MFPA</td>
<td>5.3</td>
<td>3.2</td>
</tr>
<tr>
<td>Liatest</td>
<td>1.7</td>
<td>3.7</td>
</tr>
<tr>
<td>HemosIL</td>
<td>1.9</td>
<td>5.8</td>
</tr>
<tr>
<td>STA-CLOT</td>
<td>1.3</td>
<td>3.7</td>
</tr>
<tr>
<td>PSact</td>
<td>0.7</td>
<td>4.0</td>
</tr>
<tr>
<td>CLOT S</td>
<td>2.4</td>
<td>6.4</td>
</tr>
</tbody>
</table>

STA Staclot Protein S and STA Liatest Free Protein S demonstrate good within and between run precision

Comparison of AT, PC, and PS Assays – Meijer et al

In a study of ECAT proficiency testing results between 1996-2001 (136 labs total), thrombin based AT assays, chromogenic PC, Staclot PS, and Liatest PS all showed better %CV performance compared to other tests included in the survey.

<table>
<thead>
<tr>
<th>Method</th>
<th>LCVa (%)</th>
<th>Number of laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>95% CI</td>
</tr>
<tr>
<td>Antithrombin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-IIa</td>
<td>6.7</td>
<td>2.9–17.9</td>
</tr>
<tr>
<td>Anti-Xa</td>
<td>7.3</td>
<td>4.0–41.5</td>
</tr>
<tr>
<td>Protein C activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chromogenic</td>
<td>6.8</td>
<td>3.3–24.0</td>
</tr>
<tr>
<td>Clotting</td>
<td>12.7</td>
<td>8.8–32.3</td>
</tr>
<tr>
<td>Protein S activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method A–C</td>
<td>24.9</td>
<td>14.3–45.4</td>
</tr>
<tr>
<td>Method D</td>
<td>14.0</td>
<td>4.3–44.4</td>
</tr>
<tr>
<td>Total protein S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LIA</td>
<td>6.9</td>
<td>3.5–13.3</td>
</tr>
<tr>
<td>ELISA</td>
<td>12.2</td>
<td>6.2–41.9</td>
</tr>
</tbody>
</table>
PC and PS Testing - Case Study
Thrombophilia Case Study

**Presentation:**
- 42 year old female presents with right-side chest pain followed by dyspnea, hemoptysis, and a low-grade fever. Also reported calf pain 3 days earlier.

**History**
- Craniotomy for left ophthalmic artery aneurysm repair 6 days prior, hypertension, gastroesophageal reflux, spontaneous pregnancy loss, and thyroidectomy.
- Only medication is lisinopril, no oral contraceptive use.

**Radiography:**
- Chest radiograph showed enlarged cardiac silhouette, bibasilar lung atelectis, CT scan showed bilateral lower lobe pulmonary embolism (PE) with possible right posterior basal segment infarct

# Thrombophilia Testing Case Study – Lab Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC count</td>
<td>6.9 x K/μL</td>
<td>4.0 – 11.0 x K/μL</td>
</tr>
<tr>
<td>RBC count</td>
<td>4.55 x M/μL</td>
<td>3.8 – 5.2 x M/μL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>13.3 g/dL</td>
<td>11.3 – 15.2 g/dL</td>
</tr>
<tr>
<td>Platelet count</td>
<td>324 x K/μL</td>
<td>150 – 400 x K/μL</td>
</tr>
<tr>
<td>PT</td>
<td>13.5 seconds</td>
<td>12.6 – 14.6 seconds</td>
</tr>
<tr>
<td>INR</td>
<td>1.04</td>
<td>0.9 – 1.1</td>
</tr>
<tr>
<td>aPTT</td>
<td>24 seconds</td>
<td>25 – 35 seconds</td>
</tr>
<tr>
<td>Activated protein C resistance</td>
<td>Abnormal</td>
<td>Normal</td>
</tr>
<tr>
<td>Factor V Leiden (FVL) mutation</td>
<td>Heterozygous</td>
<td>Wild type</td>
</tr>
<tr>
<td>Prothrombin G20210A mutation</td>
<td>Heterozygous</td>
<td>Wild type</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>3.5 μmol/L</td>
<td>5.0 – 12.0 μmol/L</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>IgG anticardiolipin Ab</td>
<td>&lt; 9</td>
<td>&lt; 15 GPL/mL</td>
</tr>
<tr>
<td>IgM anticardiolipin Ab</td>
<td>&lt; 9</td>
<td>&lt; 12 MPL/mL</td>
</tr>
</tbody>
</table>

Diagnosis:

- PE caused by history of untreated deep vein thrombosis (DVT). Abnormal APCR test along with heterozygosity for FVL and prothrombin G20210A mutation show elevated inherited risk for thrombophilia.
- Homocysteine was normal, but if elevated could be corrected by vitamins.
- PC/PS tests are not appropriate because acute thrombosis will reduce levels.

Treatment:

- Prolonged anticoagulation treatment for venous thromboembolism (VTE) is required.

AT, PC, PS Testing Recommendations Summarized

**AT**
- The initial screening assay should be a FXa-based or bovine FIIa-based chromogenic activity assay.
- Further tests should be carried out to distinguish HBS defects from type I and other type II defects.

**PC**
- The initial screening assay may be chromogenic which has less interferences or clotting which picks up very rare type II defects.

**PS**
- The initial screening assay should be a free PS antigenic assay because of the wide variability in PS activity assays.

- Most useful tests are AT activity, PC activity, and PS activity (free PS antigen only)
Thrombosis – Clinical Presentation

- Occurs when natural inhibitors are decreased
- May also be due to an increase in certain coagulation factors
  - VIII
  - Fibrinogen
- Patient may present with VTE (DVT/PE/stroke)
- Additional risk factors may increase the chance of having a thrombosis
  - Obesity
  - Lack of exercise
  - Smoking
  - Medications
- Patients would be treated with anticoagulants
  - UFH/LMWH
  - DOAC's
  - Warfarin
Presentation:
- 52 year old man presents in ER with dyspnea (shortness of breath), wheezing and chest tightness.
- Blood pressure is 145/88 mmHg, pulse rate >110 bpm and respiratory rate >30/min. Blood gas results reveal hypoxia (low oxygen) and cardiac exam found tachycardia (rapid heart rate).

History:
- Long history of seasonal allergy and bone fracture 1 month prior with 3 weeks immobilization. A diagnostic workup is prescribed.

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-dimer (STA-Liatest D-Di)</td>
<td>0.38 µg/mL FEU</td>
<td>&lt; 0.50 µg/mL FEU</td>
</tr>
</tbody>
</table>
## Case Study

### Lab Results

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULT</th>
<th>REFERENCE RANGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocytes (RBC)</td>
<td>5.01 x 10^{12}L</td>
<td>4.32-5.72 x 10^{12}/L</td>
</tr>
<tr>
<td>Leucocytes (WBC)</td>
<td>11.7 x 10^9/L</td>
<td>3.5-10.5 x 10^9/L</td>
</tr>
<tr>
<td>Eosinophiles</td>
<td>≥ 400 cells/μL</td>
<td>30-350 cells/μL</td>
</tr>
<tr>
<td>Platelet count</td>
<td>220 x 10^9/L</td>
<td>150-450 x 10^9/L</td>
</tr>
<tr>
<td>Hb</td>
<td>15.5g/dL</td>
<td>13.4-19.9 g/dL</td>
</tr>
<tr>
<td>MCHC</td>
<td>34g/dL</td>
<td>31-37 g/dL</td>
</tr>
<tr>
<td>MCV</td>
<td>99 fL</td>
<td>88-123 fL</td>
</tr>
<tr>
<td>D-dimer (STA-Liatest D-Di)</td>
<td>0.38 μg/mL FEU</td>
<td>&lt; 0.50 μg/mL FEU</td>
</tr>
<tr>
<td>Ig E (serum)</td>
<td>185 IU</td>
<td>100 IU</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (ESR)</td>
<td>27.8 mm/hr</td>
<td>0-22 mm/hr (men)</td>
</tr>
</tbody>
</table>
Based on the pro-inflammatory test results that the patient most likely has bronchial asthma and requires rapid acting Beta-agonists via inhalation and steroids given intravenously.

Bronchospasm and wheezing quickly relieved in response to treatment. Patient was released 2 days after.

*Negative D-dimer result excluded Pulmonary Embolism and prevented unneeded imaging from being performed.*
VTE Incidence & Importance of Early Diagnosis

350 000-900 000 VTE cases
up to 100,000 VTE-related deaths¹

over 317 000 VTE-related deaths in 6 countries of the European Union (with a total population of 454.4 million) in 2004²

Over 25,000 deaths / year

Up to 50% occur during hospitalizations

Early diagnosis of PE is crucial
Occurs within 3-7 days of onset of DVT
May be FATAL
Clinically unrecognized in most of fatal cases

¹Sources: CDC Jan 2013;
²European Heart Journal doi:10.1093/eurheartj/ehu283
What Does 100,000 Look Like?

LA Memorial Stadium

Home of the USC Trojans
Why is Exclusion of DVT/PE so Important?

Many patients who are at risk, may not know it

- Up to 40% PE fatalities have been seen by their physician in the weeks prior to their death

- 1 in 3 people with history of DVT/PE will have a recurrence within 10 years

- ~5 to 8% of the U.S. population has one of several genetic risk factors, inherited thrombophilias, in which a genetic defect increases the risk for thrombosis
  - Factor V Leiden is the most common

VTE Diagnosis: Reminder

Clinical Assessment

Pre-test probability score: identifies 3 categories of patients

- Low probability
- Moderate Probability
- High Probability

Laboratory tests

D-dimer = recognized marker for exclusion of VTE

CLINICAL + LABORATORY DIAGNOSIS

D-dimer Formation

Fibrinogen

Thrombin

Soluble Fibrin Monomer Complexes

Fibrin Monomer + fibrinopeptides

Soluble fibrin Polymer

XIIa

Fibrin clot

D-Dimer

Fibrin Degradation Products

Pre-thrombotic

Post-thrombotic
American College of Physicians PE Guideline

### PE Rule Out Criteria (PERC) Score

<table>
<thead>
<tr>
<th>Clinical Characteristic</th>
<th>Meets Criterion</th>
<th>Does Not Meet Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 50 y</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Initial heart rate &lt; 100 beats/min</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Initial oxygen saturation &gt; 94% on room air</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No unilateral leg swelling</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No hemoptysis</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No surgery or trauma within 4 wk</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No history of venous thromboembolism</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>No estrogen use</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Pretest probability with score of 0 is < 1%

## Well’s Pre-Test Probability for DVT and PE

### Well’s Pre-Test Probability for DVT

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active cancer (treatment ongoing, within previous 6 months, or palliative)</td>
<td>1</td>
</tr>
<tr>
<td>Paralysis, paresis, or recent plaster immobilization of the lower extremities</td>
<td>1</td>
</tr>
<tr>
<td>Recently bedridden for &gt; 3 days or major surgery within 4 weeks</td>
<td>1</td>
</tr>
<tr>
<td>Localized tenderness along the distribution of the deep venous system</td>
<td>1</td>
</tr>
<tr>
<td>ENTIRE leg swollen</td>
<td>1</td>
</tr>
<tr>
<td>Calf swelling &gt; 3 cm when compared with the asymptomatic leg</td>
<td>1</td>
</tr>
<tr>
<td>Pitting edema (greater in the symptomatic leg)</td>
<td>1</td>
</tr>
<tr>
<td>Collateral superficial veins (non-varicose)</td>
<td>1</td>
</tr>
<tr>
<td>Alternative diagnosis as likely as or greater than that of DVT</td>
<td>-2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

### Well’s Pre-Test Probability for PE

<table>
<thead>
<tr>
<th>Clinical Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspected DVT</td>
<td>3</td>
</tr>
<tr>
<td>Alternate diagnosis is less likely than PE</td>
<td>3</td>
</tr>
<tr>
<td>Heart Rate &gt; 100 beats/min</td>
<td>1.5</td>
</tr>
<tr>
<td>Immobilization or surgery in the previous 4 weeks</td>
<td>1.5</td>
</tr>
<tr>
<td>Previous DVT/PE</td>
<td>1.5</td>
</tr>
<tr>
<td>Hemoptysis (coughing up blood)</td>
<td>1</td>
</tr>
<tr>
<td>Malignancy (on treatment, treated in past 6 months, or palliative)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

**High pretest probability >3 points**

**Moderate pretest probability 1-2 points**

**Low pretest probability zero or negative points**

**High pretest probability >6 points**

**Moderate pretest probability 2-6 points**

**Low pretest probability <2 points**

---

Example of VTE Algorithm*

D-dimer is Sensitive BUT Not Specific for PE

Non-VTE causes of elevated D-dimer
- Cancer
- Rheumatoid arthritis
- Conditions requiring intensive care
- Advanced age (>65 years)
- Developing DIC
- Sepsis
- Inflammation
- Pregnancy

The greatest utility of D-dimer is its negative predictive value
D-dimer & VTE

- **Good news:** Almost all patients with acute disease (DVT or PE) have elevated D-dimer
- **Bad news:** BUT, not all patients with an elevated D-dimer have DVT or PE
  - Elevated D-dimer is not specific
- **Good news:** Patients with negative D-dimer are unlikely to have acute VTE
- **Great news:** *The greatest utility of D-dimer is its negative predictive value!*

The use of a qualified D-dimer assay can be used to exclude a diagnosis of VTE in patients suspected of having a VTE with a moderate or low pre-test probability.
Imaging Techniques

**Methods include**
- Compression ultrasound (CUS)
- CT of the Pulmonary Arteries (CTPA)
- V/Q Scanning
- Venography

**Imaging tests are expensive**
- CUS - $200
- V/Q, Venography, CT - > $1000

**Techs may not be available 24/7**
**Result in significant exposure to radiation**
**Final interpretation is made by a radiologist**
Compression Ultrasound

Source:
http://emedicine.medscape.com/article/1362989
Venography – Femoral Vein
CT Pulmonary Angiogram (CTPA)
V/Q Scan

- **Perfusion** – IV injection of radio-labeled albumin
- **Ventilation** – Inhalation of radio-labeled gas

Source:
Take Aways

- VTE is a significant public health concern
- Symptoms of DVT and PE are non-specific, but PE can be immediately fatal
- Diagnosis of DVT and PE is critical and presents a challenge to physicians
  - Lab and imaging tests alone are not 100% sensitive
- Pre-test probability assessment improves the sensitivity and negative predictive value of lab and imaging tests
  - Strengthens the diagnostic utility of the algorithm
- Sensitive D-dimer assays are important tools used to assess outpatients suspected of DVT and PE
Thrombosis Testing - 2018 ASH Guideline

When do we test?
Who do we test?
Diagnosis of Venous Thromboembolism

AN EDUCATIONAL SLIDE SET

AMERICAN SOCIETY OF HEMATOLOGY 2018 GUIDELINES FOR MANAGEMENT OF VENOUS THROMBOEMBOLISM
American Society of Hematology 2018 guidelines for management of venous thromboembolism: diagnosis of venous thromboembolism

Wendy Lim, Grégoire Le Gal, Shannon M. Bates, Marc Righini, Linda B. Haramati, Eddy Lang, Jeffrey Kline, Sonja Chasteen, Marcia Snyder, Payal Patel, Meha Bhatt, Parth Patel, Cody Braun, Housne Begum, Wojtek Wiercioch, Holger J. Schünemann, and Reem A. Mustafa
What these guidelines cover:

**Diagnosis of these sites of VTE:**
- PE
- DVT of lower and upper extremities
- Recurrent PE and DVT

**Using these common diagnostic tests:**
- Highly-sensitive D-dimer
- VQ scan
- Multidetector CTPA
- Compression +/- doppler US of proximal leg veins or whole leg US

All permutations of these tests were modeled for different pre-test probabilities, then compared with diagnostic studies to derive diagnostic algorithms.
Case 1: Suspected Pulmonary Embolism

70 year old female

**Past Medical History:** Emphysema, diabetes, obesity (weight 160 kg)

**Medications:** Tiotropium, salbutamol, metformin

**Seen in the Emergency Department with:** chest pain, hemoptysis x 12 hr

- No DVT symptoms, no prior VTE. No recent surgery, immobilization, or active cancer.
- Recently had viral upper respiratory infection

**Exam:** heart rate 120, oxygen saturation 93% on room air, no leg swelling or edema

**Chest X-Ray:** hyperinflation consistent with emphysema.

You determine her clinical **pre-test probability** (by Wells Score) to be **intermediate** (2.5 points)
Your patient has **intermediate pre-test probability** for PE.

Which **ONE** of the following tests would you suggest to exclude a diagnosis of PE?

A. CTPA  
B. High-sensitivity D-dimer  
C. Bilateral compression ultrasound of the legs  
D. Electrocardiogram  
E. Chest X-Ray
Your patient has **intermediate pre-test probability** for PE.

Which ONE of the following tests would you suggest to exclude a diagnosis of PE?

A. CTPA

B. High-sensitivity D-dimer

C. Bilateral compression ultrasound of the legs

D. Electrocardiogram

E. Chest X-Ray
Recommendation

The panel suggests using a strategy starting with D-dimer for excluding PE in a population with intermediate prevalence/PTP (approximately 20%), followed by VQ scan or CTPA in patients requiring additional testing (conditional recommendation, high certainty on clinical outcomes, moderate certainty on diagnostic accuracy)

Remarks:

• If D-dimer strategy is followed, a highly-sensitive D-dimer assay is required

• A negative D-dimer rules out PE, and no additional testing or anticoagulation is required
D-dimer thresholds

- D-dimer has limited utility in the following patient groups, due to high frequency of positive results with standard thresholds
  - Hospitalized patients
  - Post-surgical
  - Pregnancy

- Use of “age-adjusted” D-dimer cutoff in outpatients older than 50 years is as safe as standard cutoff and increases diagnostic utility
  - Age-adjusted cutoff = Age (years) x 10 µg/L (using D-dimer assays with a cutoff of 500 µg/L)
Your 70 year old patient’s D-dimer result is 845 µg/L (NORMAL < 500 µg/L, NORMAL age-adjusted D-Dimer < 700 µg/L).

What diagnostic test would you suggest next to exclude PE?

A. Stop investigating (positive D-dimer is diagnostic for PE)
B. Serial D-dimer test every 8 hours x 3
C. CTPA
D. VQ scan
E. Chest X-Ray
Your 70 year old patient’s D-dimer result is **845 µg/L** (NORMAL < 500 µg/L, NORMAL age-adjusted D-Dimer < 700 µg/L).

What diagnostic test would you suggest next to exclude PE?

A. Stop investigating (positive D-dimer is diagnostic for PE)
B. Serial D-dimer test every 8 hours x 3
C. **CTPA**
D. VQ scan
E. Chest X-Ray
Recommendations

• The panel recommends against using a positive D-dimer alone to diagnose PE

• Patients who are likely to have a non-diagnostic VQ scan should undergo CTPA

Remarks:

• VQ scan preferred over CTPA as subsequent test (after D-Dimer) to limit radiation exposure in patients likely to have a diagnostic scan, in centers with availability and expertise for interpretation

• However, CTPA preferred when VQ scan is not feasible
Flow chart for Diagnosis of PE in patients with intermediate PTP

CDR = Clinical Decision Rule (ie. Wells Score or Geneva Score)
# Imaging Considerations for VQ Scan and CTPA in Suspected PE

## Clinical Criteria or Concern

<table>
<thead>
<tr>
<th>Clinical Criteria or Concern</th>
<th>VQ Scan</th>
<th>CTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>At risk for reaction to contrast media requiring premedication</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Concern over radiation to female breast issue</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Renal insufficiency</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Suspected VTE recurrence or treatment failure with index PE diagnosed by VQ scan</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Suspected VTE recurrence or treatment failure with index PE diagnosed by CTPA</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>Concern over radiation to fetus (especially in first trimester)</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Minimizing risk of missed VTE at 3 months</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>Timely result required and both modalities accessible</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alternative or concomitant diagnoses actively sought (ex. cancer)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Abnormalities present on plain radiograph (hyperinflation, effusion)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Limited institutional access or expertise in Nuclear Medicine</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
ASIDE: Imagine, instead, that your patient had initially been high PTP for PE (orthopedic surgery 2 weeks ago, and signs of DVT on exam) with Wells Score of 7.

In this case, what initial diagnostic test would you suggest?

A. CTPA
B. High-sensitivity D-dimer
C. Bilateral compression ultrasound of the legs
D. Electrocardiogram
E. Chest X-Ray
ASIDE: Imagine, instead, that your patient had initially been high PTP for PE (orthopedic surgery 2 weeks ago, and signs of DVT on exam) with Wells Score of 7.

In this case, what initial diagnostic test would you suggest?

A. CTPA
B. High-sensitivity D-dimer
C. Bilateral compression ultrasound of the legs
D. Electrocardiogram
E. Chest X-Ray
Recommendation

The panel suggests using a strategy **starting with CTPA** for assessing patients suspected of having PE in a population with **high PTP (≥50%)** *(conditional recommendation, very low certainty for clinical outcomes, moderate certainty for diagnostic accuracy)*

Remarks:

- If CTPA is not feasible (contrast dye allergy, renal impairment, unavailability), VQ scan may be acceptable if non-diagnostic scans are followed by additional testing
- When clinical suspicion for PE remains high after negative initial CTPA, **additional testing** with VQ scan or proximal ultrasound of lower extremities may be considered
Flow chart for Diagnosis of PE in patients with high PTP
Case 1: Continued

• Your patient is found to have acute bilateral segmental pulmonary emboli on CTPA.

• She is started on a direct oral anticoagulant and treated for 3 months. At the end of treatment she feels back to her prior baseline.

• 3 years later, she returns with chest pain, dyspnea, and signs of right leg DVT. She has been having hemoptysis and is tachycardic. You feel that she is “high (likely)” PTP for recurrent PE (Wells score of 7)
You are concerned about the possibility of recurrent PE. You feel that your patient has highly/likely PTP.

What test would you suggest to exclude recurrent PE?

A. CTPA
B. High-sensitivity D-dimer
C. Bilateral compression ultrasound of the legs
D. Electrocardiogram
E. Chest X-Ray
You are concerned about the possibility of recurrent PE. You feel that your patient has highly/likely PTP.

What test would you suggest to exclude recurrent PE?

A. CTPA
B. High-sensitivity D-dimer
C. Bilateral compression ultrasound of the legs
D. Electrocardiogram
E. Chest X-Ray
Recommendation

• Patients with a **positive D-dimer, or those who have a likely PTP** should undergo **CTPA** (conditional recommendation, low certainty for clinical outcomes, moderate certainty on diagnostic accuracy)

• The panel suggests using a strategy **starting with D-dimer** for excluding recurrent PE in a population with **unlikely PTP**.

In studies examining this diagnostic strategy for recurrent PE, **the Wells and Geneva Scores** were used as clinical prediction rules.

**Note:** they have not been specifically validated in patients with suspected recurrent PE.

If prior imaging is available, **comparison of previous and current imaging** warranted to determine if findings are new and represent recurrent PE.

Mos Thromb Res 2014
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Flow chart for Diagnosis of **recurrent PE**

**Case 1: Continued**

- Your patient’s PTP is high/likely, so you arrange for CTPA
- The CTPA does not demonstrate PE, and recurrent PE is ruled out
Case 1: Summary

In patients with low or intermediate PTP for PE, a high-sensitivity D-dimer, if negative, can safely exclude PE with no additional testing.

CTPA is preferred over VQ scan in individuals who are likely to have a non-diagnostic VQ result, including patients who are elderly or who have pre-existing lung disease.

Patients with suspected recurrent PE should be stratified into likely or unlikely PTP to determine subsequent testing, although clinical prediction rules have not been extensively validated for recurrent PE.
Case 2: Suspected Deep Vein Thrombosis

45 year old male

Past Medical History: Hypertension, lung cancer
Medications: Ramipril, amlodipine, chemotherapy (cisplatin/gemcitabine)

Seen in the Emergency Department with: left calf pain and swelling x 48 hr

- No recent surgery or immobilization
- Receiving chemotherapy
- No chest pain, dyspnea
- No varicose veins

Exam: heart rate 80, oxygen saturation 97% on room air.
- Left calf circumference 5 cm greater than right calf
- Localized tenderness along venous system
- Pitting edema in left leg

You determine his clinical pre-test probability to be high (by Wells Score = 4)
Your patient with high PTP undergoes a left leg proximal compression ultrasound. The ultrasound does not demonstrate evidence of DVT.

Which diagnostic test would you suggest next?

A. Stop investigations as his ultrasound is negative
B. Serial proximal compression ultrasound within one week
C. High-sensitivity D-dimer
D. Venography
E. CTPA
Your patient with high PTP undergoes a left leg proximal compression ultrasound. The ultrasound does not demonstrate evidence of DVT.

Which diagnostic test would you suggest next?

A. Stop investigations as his ultrasound is negative
B. Serial proximal compression ultrasound within one week
C. High-sensitivity D-dimer
D. Venography
E. CTPA
**Recommendation**

- The panel suggests using a strategy **starting with proximal lower extremity or whole leg ultrasound** for assessing patients suspected of having DVT in a population with **high prevalence/PTP** (≥50%).

- **This should be followed by serial ultrasound if the initial ultrasound is negative and no alternative diagnosis is identified** (conditional recommendation, very low certainty on clinical outcomes, high certainty on diagnostic accuracy)

**Remarks:**

- If a two-level clinical decision rule (ie. likely vs. unlikely) is utilized, this recommendation corresponds to the “likely DVT” category
For patients at **high PTP**, a single proximal or whole leg US is not sufficient to rule out DVT.

Subsequent testing with serial US is required.
By contrast, in patients with **low PTP for DVT**, D-dimer recommended as first diagnostic test to exclude DVT.
Case 2: Continued

• Your patient, whose PTP was high, has a serial proximal ultrasound in 7 days. This ultrasound demonstrates occlusive DVT within the left popliteal and superficial femoral veins.

• Your patient is started on anticoagulation with LMWH and you arrange for follow up with in thrombosis clinic.
Case 2: Four months later

- Four months later he remains compliant on full-dose anticoagulation with LMWH.

- Unfortunately his lung cancer is progressing despite chemotherapy, with worsening metastatic disease.

- He presents to hospital with swelling and tightness in his left (ipsilateral) calf. There is localized pain and unilateral edema. You feel his PTP for recurrent DVT is likely (Wells Score of 4).
Your patient who sustained DVT 4 months ago presents with recurrent leg symptoms and likely PTP.

What diagnostic test would you suggest at this point?

A. CT scan of the abdomen
B. High-sensitivity D-dimer
C. Venography
D. Left leg compression ultrasound
Your patient who sustained DVT 4 months ago presents with recurrent leg symptoms and likely PTP.

What diagnostic test would you suggest at this point?

A. CT scan of the abdomen
B. High-sensitivity D-dimer
C. Venography
D. Left leg compression ultrasound
In patients with a prior history of DVT, what is the optimal diagnostic strategy to evaluate for suspected recurrent DVT?

Recommendation

- **Patients with positive D-dimer or those who have likely PTP:** should undergo **proximal lower extremity ultrasound** *(conditional recommendation, low certainty)*

- **In a population with unlikely PTP:** the panel suggests using a strategy **starting with D-dimer** for excluding recurrent DVT

If prior imaging is available, **comparison of the previous and current imaging** is warranted to determine if the findings are new and represent recurrent PE

Ultrasound findings of recurrent DVT may include involvement of new venous segment or increase in non-compressibility of >4mm.
Flow chart for Diagnosis of recurrent DVT

In studies assessing this diagnostic strategy for suspected recurrent DVT, a modified Wells score was used to assess clinical probability.
Case 2: Conclusion

• As your patient has likely PTP, he undergoes a compression US which reveals a non-occlusive left leg popliteal vein thrombosis, which is improved compared with his previous DVT

• He is advised that he does not have recurrent DVT, and he remains on LMWH anticoagulant therapy
Case 2: Summary

In patients with high PTP and negative initial compression ultrasound, serial imaging is indicated to exclude DVT.

In patients with low PTP, D-dimer is the first recommended diagnostic test to exclude DVT.

When assessing for recurrence of DVT, comparison of prior and current imaging is warranted to determine if radiographic findings are old or represent recurrence.
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See more about the ASH VTE guidelines at www.hematology.org/vte