Clotting Disorders: What You Need To Know

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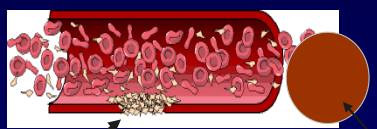
Pre-test

- a. Factor V Leiden is the most recently discovered hereditary or genetic risk factor for thrombosis.
  - 1.True__ / False__
- b. Anticardiolipin antibodies target the negatively charged phospholipid cardiolipin.
  - 1.True__ / False__

Approach To thrombotic Disorders

- Hemostasis
  - - Normal mechanism of clotting of blood
- Thrombosis = clot in the wrong place
  - = hemostasis gone wrong

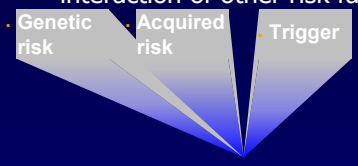


good clot

bad clot

Thrombosis Risk

- Thrombosis Potential: gene defects confer risk
- - but do not result in thrombosis w/o interaction of other risk factors



Thrombophilia: Clinical Characteristics

- Thrombophilia- refers to inherited risk factors for VTE
- Family history of venous thrombosis
- Younger age of onset
  - (Rule of thumb AGE <45-50)
- Lesser environmental stimulus
- Thrombus formation often in unusual sites
- Woman w/ history of multiple abortions, stillbirth, or both

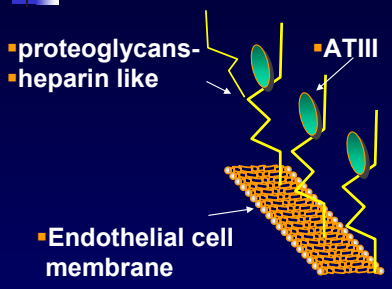
Causes Of Thrombophilia (hypercoagulable State)

Frequent or Well Established
Anti-thrombin III
Protein C deficiency
Protein S deficiency
Factor V Leiden ([Gln506]factor V) or APC Resistance
Prothrombin Gene Mutation (FII20210 G to A)
Hyperhomocysteinemia

AT III Deficiency

- Antithrombin III (AT) was the first coagulation protein associated with thrombophilia
  - In 1965- Egeberg described a Norwegian family with recurrent thrombosis and AT levels of 40-50%
- AT is a single chain glycoprotein of 60kd.
- Synthesized by the liver, with plasma 1/2 life of 48hrs; Plasma concentration - 140mcg/ml
- Gene is located on Chr 1(q23-25)

AT III And Natural Proteoglycans

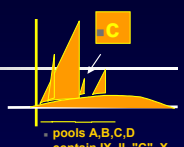


AT III Deficiency

- AT deficiency is inherited in an autosomal dominant manner, with heterozygotes being affected and having levels approximately 40-70% of normals
- AT deficiency is divided into 2 types:
- Type I - both antigen and activity are low
- Type II- low activity with normal antigen
  - Type II has been further subdivided into 3 subtypes
    - Those affecting the reactive site
    - Those affecting the heparin binding site
    - Those with both effects

Protein C

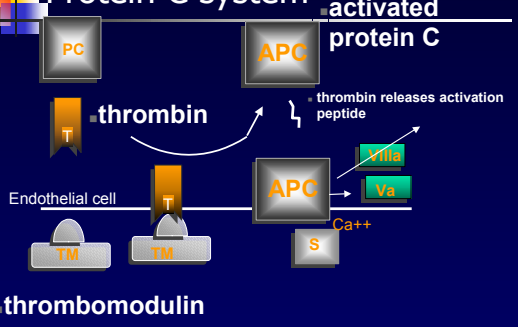
- Discovered as new Vit K dependent protein in 1976
  - Johan Stenflo. JBC 251:355,1976
- Function described 1979- a serine protease
  - Walker, sexton,Esmon. Biochem Biophys Acta 571:333,1979
- 62kd protein, 3-5 mcg/ml
- Gene on -chromosome 2
  - (Q13-4)



Protein S

- Discovered in 1979
  - DiScipio RG. Protein S. Biochemistry 16: 698-706, 1977
  - Named after City of discovery-"Seattle"
- 69kD; 20-25 mcg/ml
- synthesized in liver
- Association with Thrombosis recognized in 1984
  - Comp, Esmon. N Eng J Med 311:1525, 1984

Protein C system



Protein S

- Protein S, in contrast to other factors, circulates in 2 forms-
  - 1) majority of S is bound to a complement protein, c4b-binding protein (c4bbp);
  - 2) remainder is free S
- Approx. 30% to 40% total protein S in free state

Protein S

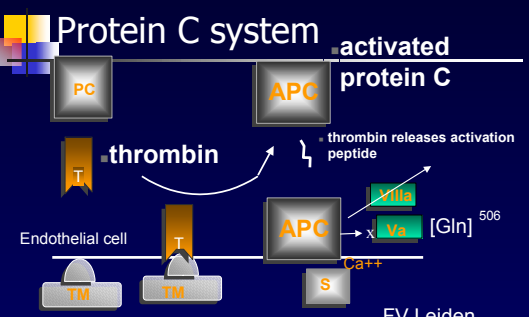
- Only free protein S is capable of acting as a cofactor in the protein C system
- 3 types of deficiency
  - *Type I
    - low total & free S-Ag
  - Type II
    - abnormal function
    - Normal total & free S-Ag
  - *Type III-
    - low free S-Ag
    - Normal total S-Ag

* **most common**

APC Resistance

- In 1993, it was found that plasmas from individuals with inherited thrombophilia were resistant to the anticoagulant effect of activated protein C (APC)
- Three labs idependently identified the problem as a mutation in factor V(G1691A)
  - Replacement of arginine in residue 506 by glutamine
    - Dahlback B. Haemostasis. 1994;24:139
    - Bertina RM nature. 1994;369:64
    - Voorberg J lancet. 1994;343:1535
  - Arg506Gln mutation renders FVa resistant to APC
  - Arg506 and arg306 are the cleavage sites of FV by APC
- Termed FACTOR V LEIDEN

Protein C system



FV Leiden
10 x less
inactivation

Prothrombin Gene Mutation

- Recently investigators went hunting for a new cause of hypercoagulability
- They directly examined the prothrombin gene (FII)
  - They looked for mutations in the entire gene in selected patients with a documented familial history of VTE.

Poort SR Blood. 1996;88:3698–3703.

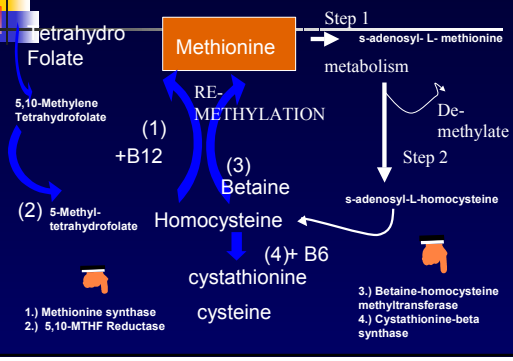
Prothrombin Gene Mutation

- The researchers evaluated
  - the coding region (exons)
  - and the regulatory regions (5' and 3' untranslated flanking regions)
  - of the gene by PCR
  - They found a single nucleotide G to A change in the 3' flanking region at position 20210 in patients with VTE
- Termed Prothrombin or F II (G20210A) or 20210A

Hyperhomocysteinemia

- An abnormal increase in Homocysteine
  - A sulfhydryl amino acid derived from metabolic conversion of methionine
- Caused by hereditary defects in one of several enzymes in the metabolic pathway of homocysteine
- Or acquired deficiencies of vitamins B12, B6 , folic acid

Homocysteine Metabolism



Hyperhomocysteinemia: Thrombosis

- Thermolabile variant of MTHFR
  - The most common form of genetic hyperhomocysteinemia results from production of a thermolabile variant of methylene tetrahydrofolate reductase (MTHFR) with reduced enzymatic activity (T mutation)
  - The gene encoding for this variant contains an alanine-to-valine substitution at amino acid 677 (C677T)
  - Homozygosity for the thermolabile variant of MTHFR (TT genotype) is a relatively common cause of mildly elevated plasma homocysteine levels in the general population, often occurring in association with low serum folate levels

Frequency: Inherited Hypercoagulable States

Syndrome	General Pop (%)	Unselect VTE (%)	Select VTE (%)
AT3 def	0.02	1.1	0.5-4.9
Protein C	0.14	3.2	1.4-8.6
Protein S	-	2.2	1.4-7.5
Factor V Leiden	3.6-6	21	10-64
Prothrombin Gene Mutation	2-3	6.2	18
Hyperhomocysteinemia	-	-	10

Acquired Risk Factors for VTE (hypercoagulable State)

CLINICAL FACTORS
Anti-cardiolipin antibody, or lupus anticoagulant
Cancer
Myeloproliferative disorders, PNH
Surgery, Trauma, CHF, etc.
Nephrotic Syndrome
Pregnancy, OCP, HRT
PATIENT FACTORS
Obesity, Older age, Immobilization
Hyperhomocysteinemia (aquired)

Lupus Anticoagulant

- 1952- Conley and Hartmann recognize an inhibitor of clotting tests in 2 pts with SLE
- 1974-lupus inhibitor present in pts with BFP-STS
- 1977-Feinstein and Rappaport term it the "lupus anticoagulant (LA) "
- 1980-Thiagarajan shows IgM Moab LA reacts w/ neg charged PLs

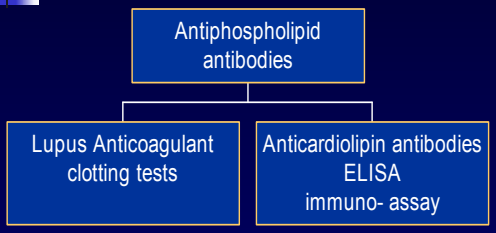
Lupus Anticoagulant (LA)

- Definition: LACs are Immunoglobulins that interfere with in vitro phospholipid coagulation tests
- The minimum lab requirements:
  - Prolongation of at least one PL dependent clotting test
    - (Pt, PTT, DRVVT)
  - Demonstration that this prolongation is due to an endogenous inhibitor

Diagnosis Of Lupus Anticoagulant (LAC)

- LACs are important because they are common causes of increased APTT and are associated with thrombosis

Antiphospholipid Antibodies



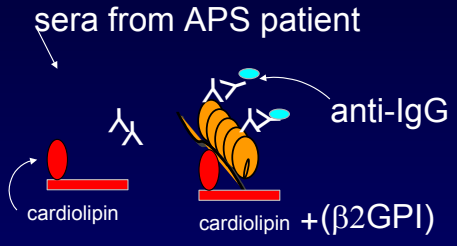
Cardiolipin Is Not the Antigen for aCL

- 3 groups in 1990 independently showed that purified aCL abs did not bind to cardiolipin in the absence of serum or plasma
- A normal plasma protein beta- 2-glycoprotein I (b2GPI) was found to be the antigen responsible for ab binding

Beta-2-glycoprotein I (b2GPI)

- Beta-2-glycoprotein I (b2GPI)
  - a single chain polypeptide w 326 aa of 50kd mw
  - plasma concentration ~200mcg/ml
  - member of the complement control family -5 consensus repeats
- physiologic function not known

aCL ELISA



aCL ELISA

- Most if not all "anticardiolipin" antibodies in APS bind to epitopes on B2GPI- not CL
- Recent data indicate abs bind to B2GPI alone- even w/out phospholipid
- Authentic anticardiolipin abs occur in infectious disease, syphilis, normals

Thrombophilia Testing

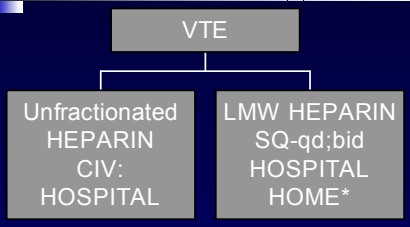
Disorder		Interfering substances
AT 3-function		acute thrombosis, heparin, liver disease, nephrotic, DIC, estrogen replacement (HRT)
Protein C-Antigen		coumadin, acute thrombosis
Protein C-function		coumadin, anticoagulants, acute thrombosis
Protein S-antigen		coumadin, acute thrombosis, pregnancy, sickle cell anemia
Protein S-function		coumadin, anticoagulants, acute thrombosis, pregnancy*

- * Am J Obstet Gynecol 1996;175:657-60

Thrombophilia Testing cont'd

Disorder		Interfering substances
APC resistance		Heparin, Lupus Anticoag, FVIII, deficiencies, OCP, Pregnancy
Factor V Leiden		none
Prothrombin Gene Mutation		none
Hyperhomocysteinemia		food- fasting needed; tricky test
Lupus Anticoagulant		Heparin
anti-Cardiolipin abs (aCL)		low titer skewed normal; infections (HIV)

Updated Treatment VTE: 2004



Key Principles in Management of VTE with UH

- IV bolus, followed by
- CIV infusion
- Monitor aPTT 6hrs p bolus & CIV started, repeat in 6hrs
- Standard protocol for dose adjustment
- Therapeutic aPTT range
  - - achieve 1st 24 hrs
- Minimum 5 days Heparin Rx
- Minimum 2 days therapeutic PT before stopping heparin

Management

- Indefinite anticoagulant therapy
- considered in patients without an obvious laboratory abnormality
- if the episodes are idiopathic, multiple, occur at different sites, are life-threatening, or occur in an unusual site such as the IVC, cerebral veins, or inferior mesenteric vein."

Management

- Asymptomatic carriers and those with previous episodes of venous thrombosis
- Prophylaxis
  - that is limited to a defined period of risk (e.g., an operation)
- Prophylaxis with either adjusted dose heparin or oral anticoagulants
  - during surgery or enforced bed rest or immobilization

Duration Of Anticoagulation

Table 2. Suggested Duration of Anticoagulation after Venous Thromboembolism.

Type of Event or Condition	Duration	Source of Evidence
No identified thrombophilic abnormalities		
First event, distal deep-vein thrombosis, provoked by temporary risk factor	6 Wk	Schulman et al.[*]
First event, distal deep-vein thrombosis with idiopathic thrombosis or pulmonary embolism, proximal deep-vein thrombosis or pulmonary embolism	≥6 Mo[*]	Ridker et al.,[**] Kearon et al.,[**] Schulman et al.,[**] Pinede et al.[**]
As above, with increased risk of bleeding	3 Mo	
Single life-threatening event	≥12 Mo[*]	
First event, active cancer	Until cancer resolved	Hutten et al.[**]
Second event, contralateral deep-vein thrombosis	≥6 Mo (as a first event)	Schulman et al.[**]
Second event, ipsilateral or pulmonary embolism	≥12 Mo[*]	Schulman et al.,[**] Prandoni et al.[**]
Third or subsequent event	Indefinite	
Thrombophilic defects identified†		
Deficiency of antithrombin	Indefinite	Case series, Lane et al.[**]
Deficiency of protein C or protein S	≥12 Mo	van den Belt et al.[**]
Homozygous form of thrombophilic defect	Indefinite‡	Case reports, Procare Group[**]
Heterozygous for two thrombophilic defects	Indefinite	De Stefano et al.[**]
Antiphospholipid-antibody syndrome	Years	Khamashta et al.[**]
Hyperhomocysteinemia	Variable§	Schnyder et al.[**]
Elevated factor VIII activity (>2.3 IU/ml)	≥6 Mo	Kyrle et al.[**]
Factor V Leiden mutation, heterozygous	As without	Lindmarker et al.[**]
Prothrombin polymorphism, heterozygous	As without	Lindmarker et al.[**]
Life-threatening event and any defect	Indefinite	Lane et al.[**]

* Some recent data[**,**] suggest that the benefit of indefinite anticoagulation outweighs the risk after isolated venous thrombosis, even without identifiable thrombophilia.
† The presence of the defects listed is independent of provocation of the event by a temporary or permanent risk factor, whenever the thrombophilic defect is permanent.
‡ This recommendation may not pertain to the homozygous form of the prothrombin polymorphism.
§ The duration depends on factors other than the biochemical defect. Hyperhomocysteinemia does not affect the duration of anticoagulation, but after anticoagulation is completed, treatment with B vitamins should probably be given.

N Engl J Med 2003;349:675-83

DISCUSSION AND CLOSE