Thrombophilia

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Function of Hemostasis

- Stop bleeding.
  - Primary and secondary hemostasis.
- Maintain blood in fluid state.
  - Control mechanisms of coagulation.
- Removal of clot after healing of injury.
  - Fibrinolytic system.
Primary Hemostasis

ADHESION

- Platelet
- GPIb
- VWF
- GPIIb/IIIa
- Collagen binding site
- Exposed collagen at site of injury
- Endothelial cells

AGGREGATION

- Platelet
- GPIb
- VWF
- GPIIb/IIIa
- GPIIb/IIIa
Coagulation Cascade
Control mechanism of coagulation

- Naturally occurring inhibitor (TFPI)
- Serine protease inhibitors
  - Antithrombin
  - Heparin and heparin cofactor
- Protein C system
Definition of Thrombophilia

British committee for standard in hematology 1999: Disorders of the hemostatic mechanisms which are likely to predispose to thrombosis.

In North America: Clinicians use it to describe patients who have developed venous thrombosis either spontaneously or due to recognized stimulus, patients who have recurrent venous thrombotic events and patients who develop venous thrombosis at early life.
Why Perform Thrombophilia Testing?

- To provide knowledge of the pathologic basis of the thrombosis and provide the opportunity to communicate etiologic factors to patients.
- To influence duration and intensity of therapy during a thrombotic episode.
- To offer thrombosis prophylaxis for high risk patients during periods of potential increased thrombosis stimulus.
- To alert patient’s kindred to the presence of inherited risk factors.
- To determine the need for alternative laboratory testing when condition affects primary testing mode.
Reasons to Order Laboratory Workup for Thrombophilia

- Venous thrombosis before 40-50 years age.
- Unprovoked thrombosis at any age.
- Recurrent thrombosis at any age.
- Thrombosis at unusual sites.
- Positive family history of thrombosis.
- Thrombosis secondary to pregnancy, oral contraceptives, or hormone replacement therapy.
- Unexplained abnormal laboratory test such as prolonged PTT.
Heritable Thrombophilia

Limited number of genetic variants are proven to be independent risk factors for venous thrombosis:

- Mutations in the genes encoding the natural anticoagulants:
  - Antithrombin
  - Protein C
  - Protein S

- Mutations in the genes encoding the clotting factors:
  - Fibrinogen
  - Prothrombin
  - Factor V

- Mutation in the MTHFR enzyme causing hyperhomocysteinemia
Antithrombin

- The most important physiologic inhibitor of thrombin.
- Its action is accelerated at least 2000-fold in the presence of heparin.
- Deficiency is associated with 8-fold increased risk of thrombosis.
- 1 in 2-5000 in normal population
- 0.5-1% of thrombotic patients
- Gene on long arm of chromosome 1.
- Several mutations have been reported some of them in independent reports in different families.
- Functional assay to determine antithrombin activity
- Ag assay when activity is consistently low
- Mutation detection is not applicable for routine testing.
Proteins C and S

Protein C

↓

Thrombin-thrombomodulin complex

APC

↓

Protein S

Proteolytically inactivates Factors Va and VIIIa
Proteins C and S deficiencies are associated with an 8-fold increased risk of thrombosis. Genes are located on chromosome 2 and 3. A wide variety of mutations were reported in both genes causing deficiencies of the proteins. Activity of both proteins is evaluated by functional assays. Protein C Ag and free and total protein S Ag should be measured if activity is consistently low. Mutation detection is not applicable for routine testing.
Activated Protein C Resistance (APCR)

Protein C

\[ \downarrow \]

Thrombin-thrombomodulin complex

APC

\[ \downarrow \]

Protein S

Proteolytic inactivation of FVa
The most frequent laboratory abnormality in patients with history of thrombosis.

**FV Leiden mutation (R506Q)**
- Heterozygous 2-8 fold increased risk of thrombosis
- Homozygous 80-100 fold increased risk of thrombosis
- Oral contraceptive and heterozygosity increase thrombotic risk in female 30-50 fold
- Heterozygosity in pregnancy also increase risk of thrombosis
- Present in 5% normal Caucasians
- Explains 20-40% thrombotic Caucasians

**Other mutations in FV** (e.g. FV Cambridge and FV Hong Kong in codon 306).
Prothrombin Mutation G20210A

- Reported by Bertina group 1996
- Heterozygous 2-4 fold increase risk of thrombosis
- 2% in normal population
- 6-7% unselected patients with DVT
- 18% in selected patients with history of venous thrombosis
- Coinheritance occur with FV Leiden in 1% of thrombophilia patients. This result in thrombotic event at very young age (20-25 years of age).
- No functional assay to detect the phenotypic variant
- DNA-based procedure is required.
Hyperhomocysteinemia

- Increased blood-level of homocystein was reported as risk factor for arterial and venous thrombosis.
- 2-fold increase risk of thrombosis.
- 4-fold increase risk of thrombosis in conjunction with oral contraceptive.
- Mutations in enzymes responsible of homocystein metabolic pathway (MTHFR).
- C677T in the MTHFR gene.
- Heterozygous in 40% and homozygous in 10% normal Caucasian population.
Metabolism of homocysteine
# Summary of Heritable Thrombophilia

<table>
<thead>
<tr>
<th>Factor</th>
<th>General Population</th>
<th>People With Thrombosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>APCR</td>
<td>3-8% Caucasians</td>
<td>20-40%</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>2-3% Caucasians</td>
<td>4-8%</td>
</tr>
<tr>
<td>Antithrombin def.</td>
<td>1 in 2-5000</td>
<td>1%</td>
</tr>
<tr>
<td>Protein C def.</td>
<td>1 in 300</td>
<td>2-10%</td>
</tr>
<tr>
<td>Protein S def.</td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>Hyperhomocyst.</td>
<td>11%</td>
<td>-</td>
</tr>
</tbody>
</table>
Recommended Molecular Testing in Thrombophilia Investigation

- FV Leiden Mutation
- Prothrombin G20210A mutation
- MTHFR C677T mutation

Are these applicable in our population?
PCR Amplification for MTHFR

198bp
Method Used for MTHFR Mutation Detection

MTHFR Gene

PCR product (198bp)

PCR product is digested with *Hinfl* restriction enzyme

198 bp

In normal MTHFR

In Homozygous for the mutation

In Heterozygous for the mutation
Diagrammatic representation \textit{Hinfl} Analysis

<table>
<thead>
<tr>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>198bp</td>
<td>198bp</td>
</tr>
<tr>
<td></td>
<td>175bp</td>
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</tr>
</tbody>
</table>
Restriction Enzyme Analysis for MTHFR Mutation

175bp 198bp
Method Used for FV Leiden Mutation Detection

FV Gene

PCR product (143bp)

PCR product is digested with MnlI restriction enzyme

In normal FV

81 bp  37 bp  25 bp

In Homozygous for the mutation

118 bp  25 bp

In Heterozygous for the mutation

81 bp  37 bp  25 bp

118 bp  25 bp
PCR Amplification for FV Leiden

143 bp
## Diagrammatic representation MnII Analysis

<table>
<thead>
<tr>
<th>Homozygous</th>
<th>Heterozygous</th>
<th>Normal</th>
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<tbody>
<tr>
<td></td>
<td>118bp</td>
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<tr>
<td></td>
<td></td>
<td>81bp</td>
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<tr>
<td></td>
<td></td>
<td>37bp</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25bp</td>
</tr>
</tbody>
</table>
Restriction Enzyme Analysis for FV Leiden Mutation

- UP
- Heterozygous
- 118 bp
- 81 bp
- 37 bp
- 25 bp
Thank You