Coagulation Cascade

Intrinsic Pathway

“Contact
Activation”

"TF Pathway"

Extrinsic Pathway

Tissue Factor + VII

XIIIa

Fibrinogen

TF-VIIa

IX

Ca2+

PL

Prekallikrein

HMW

Kininogen

XIIa

XIIa

XI

XIII

“TF Pathway”

PL, Ca2+

(Tenase)

VIIIa

IXa

Xa

Va

VIIIa

PL, Ca2+

(Prothrombinase)

Prothrombin

Common Pathway

Thrombin

Fibrin Monomer

Fibrin Polymer

XIIIa

Anticoagulation proteins:
Protein C, Protein S,
Antithrombin III, TFPI
APC Resistance

- Common in the general population
- Most common cause of hereditary thrombophilia
- Can be hereditary or acquired
- APC Resistance alone is not a significant risk factor. Having APC Resistance combined with other risk factors, however, greatly increases risk of thrombosis
ANTICOAGULANT RESPONSE TO APC

APC resistance phenotype

• A poor anticoagulant response to activated protein C (APC).
• In an APC R patient, there is not as much inactivation of coagulation.
INACTIVATION OF NORMAL FVa

APC cleavage sites

306  506  679

FVa heavy chain

Ca²⁺

FVa light chain

• APC cleaves sites on the heavy chain, inactivating FVa and helping to prevent too much thrombin activation.
• Cleaves at the 306, 679 and 506 positions.
Arg to Glu Mutation results in a 10-fold lower inactivation rate of FVa, i.e. FVa molecule isn’t allowing APC to do its job of inactivating FVa and ultimately inhibiting thrombin generation.

FV Leiden Mutation
- Accounts for approx. 90% of APC Resistance
- Prevalent in about 2 – 13% of general population
- Accounts for about 20 – 60% of VTE cases
- Heterozygotes for FV Leiden have 2 – 5 fold increased thrombotic risk
GENETIC AND ACQUIRED RISKS

Genetic risk factors:
APC resistance (FV:Q^{506}, FV Leiden)

Acquired risk factors:
Surgery, Pregnancy and Oral Contraceptive Pills / Patch
Account for about 5 – 10% of APC resistance
TESTING FOR APC RESISTANCE

• “Gold standard” is an APTT based clotting assay.
• Two APTT tests are run: one with CaCl2 (“Baseline clotting time”) and one with an excess of APC and CaCl2 (“Activated clotting time”).
• Record the clotting times and calculate the ratio.
• In a normal patient, this excess APC will cause inactivation of FVa at a higher rate, meaning less thrombin generation, prolonged clotting time, and higher ratio between basal and APC clotting times.
• In an abnormal patient, however, even if you add that excess APC, FVa is not being inactivated as much, so you don’t see that prolongation of APC clotting time.
• Therefore, the ratio between basal and APC clotting times is not as high as it would be in a normal patient.

• By diluting the sample 1:4 in FV-deficient plasma, you test for FV Leiden. This also allows testing of samples containing heparin or warfarin.
**APTT-based APC Resistance Assays**

Sample Plasma + V DEF Plasma = Prediluted Plasma

- 1 vol. Prediluted Plasma
- Incubate 5 min. 37°C

- 1 vol. APTT
- +

- 1 vol. CaCl₂
- +

- 1 vol. APC/CaCl₂

Record time for clot formation
APC RESISTANCE: INTERPRETATION OF RESULTS

- APC- ratio = $\frac{\text{Clot time APC/CaCl}_2}{\text{Clot time CaCl}_2}$

- APC Resistance is indicated when the APC ratio is below or equal to the calculated cut-off value.

- APC R V ratio below the calculated cut-off is due to presence of the factor V:Q506 mutation.
APTT-based APC Resistance Assays

• Benefit:
  • Offers genotypic information for clinical decision-making

• Utility:
  • For factor V:Q\textsuperscript{506} mutation screening
  • Ratio at or below cut-off may be confirmed with genetic test

• Features:
  • Unsurpassed sensitivity for the factor V:Q\textsuperscript{506} mutation and close to 100% specificity
  • Applicable to anticoagulant treated patients
  • Economical alternative to genetic testing
APC Resistance

Clear discrimination between normals, heterozygotes, and homozygotes is achieved with the APTT-based screening assay.
COATEST® APC RESISTANCE V

The Gold Standard for APC Resistance Testing!