LABORATORY APPROACH TO
BLEEDING DISORDERS

DR NISHANTH
PG 1ST YEAR
DEPARTMENT OF PATHOLOGY
WHEN IS THE LAB REQUIRED TO INVESTIGATE FOR A POSSIBLE BLEEDING DISORDER?

- Clinically suspected bleeding tendency
  
  H/O bleed following trivial trauma

- Following up an abnormal first line test
  
  Incidental/ intended testing

- Intractable bleed
  
  Surgical/ Non surgical trauma
BLEEDING DISORDERS

DEFECT IN

PRIMARY HAEMOSTASIS

VASCULAR PLATELETS

SECONDARY HEMOSTASIS

COAGULATION
SCREENING TESTS
SCREENING TESTS

• Simple to perform, rapid
• Assess the integrity of primary or secondary haemostasis
• Do not pinpoint the nature of defect

- Complete blood count and blood smear
- Platelet count
- Mean platelet volume
- Clotting time
- Prothrombin time
- Activated partial thromboplastin time
- Thrombin time
- Platelet function analyser-100
PRIMARY HAEMOSTASIS
### SCREENING TEST FOR PRIMARY HAEMOSTASIS

<table>
<thead>
<tr>
<th>Test</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Bleeding time</td>
<td>Platelet and vascular phases</td>
</tr>
<tr>
<td>• PFA-100 system</td>
<td>Platelet function</td>
</tr>
<tr>
<td>• Platelet count</td>
<td>Quantitation of platelets</td>
</tr>
<tr>
<td>• Blood smear</td>
<td>1] Quantitative and morphological abnormalities</td>
</tr>
<tr>
<td></td>
<td>2] Detection of underlying haematological disorder</td>
</tr>
</tbody>
</table>
SCREENING TEST FOR PRIMARY HAEMOSTASIS

- **Bleeding time**: Normal 2 - 7 minutes
- **Platelet count**: Normal 1.5 - 4 lakh/cmm
EVALUATION OF PROLONGED BLEEDING TIME

Bleeding time prolonged

Platelet count

Normal

Von Willebrand disease, Platelet function disorders, Vascular disorders, Afibrinogenemia

Low

Bone marrow examination

Megakaryocytes N / ↑

Increased platelets destruction

Megakaryocytes absent

Decreased production of platelets
EVALUATION OF THROMBOCYTOPENIA

- Reticulocyte count
- Schistocytes
- MIHA
- Coagulation profile
- Normal
- ABNORMAL
- DIC
- PSEUDOTHROMBOCYTOPENIA
- Bone marrow examination
- Acute leukemia, MDS
- Aplastic anemia, Hypersplenism
- ITP, DRUGS, INFECTION
- Isolated thrombocytopenia
- Platelet satellitism
- THROMBOCYTOPENIA
- BLOOD SMEARS
PLATELET FUNCTIONAL ANALYSER-100 (PFA-100)

- Used to assess platelet adhesion and aggregation
- Normal closure: 1-3 min
- If results abnormal then platelets aggregation studies is definitive
SECONDARY HEMOSTASIS
COLLECTION OF BLOOD SAMPLE FOR COAGULATION STUDIES

• Venous blood sample.
• Should not be collected from indwelling catheter – heparin.
• Glass syringe/glass tube should not be used – contact factor.
• Anticoagulant - Aqueous trisodium citrate(3.2%)
• Proportion blood to anticoagulant - 9:1
• Platelet poor plasma – centrifugation at 3000 rpm for 15 to 30 mins.
• Studies done within 2 hours of collection of sample
MECHANISM OF NORMAL CLOT FORMATION

Intrinsic pathway:
- Prothrombin → Thrombin → Fibrinogen → Fibrin

Extrinsic pathway:
- Xa + Va → Fibrin monomer → Polymerization → FIBRIN PLUG
**SCREENING TEST FOR SECONDARY HAEMOSTASIS**

<table>
<thead>
<tr>
<th>Test</th>
<th>Assessment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotting time (CT)</td>
<td>Crude test of coagulation phase</td>
</tr>
<tr>
<td>Prothrombin time (PT)</td>
<td>Extrinsic and common pathway</td>
</tr>
<tr>
<td>Activated partial thromboplastin</td>
<td>intrinsic and common pathway</td>
</tr>
<tr>
<td>time (APTT)</td>
<td></td>
</tr>
</tbody>
</table>

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PROTHROMBIN TIME (PT)

- Normal range: 11-16 sec
- Prolonged in:
  1) Treatment with oral anticoagulants
  2) Liver diseases
  3) Vitamin K deficiency
  4) DIC
  5) Inherited deficiency of extrinsic or common pathway
**Prothrombin**

**Fibrinogen**

**Fibrin**

**Fibrin monomer**

**Fibrin plug**

**APTT**

**Intrinsic pathway**

**XIIa, XIa, IXa, VIIIa**

**VIIa**

**Extrinsic pathway**

**PT**

**Fibrinogen conc.**

(Thrombin time test)

(polymerization)

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ACTIVATED PARTIAL THROMBOPLASTIN TIME - APTT

• Normal range: 30 to 40 seconds
• Prolonged in 1) Hemophilia A or B
  2) Circulating inhibitors
  3) DIC
  4) Heparin therapy
  5) Liver disease
  6) Vitamin k deficiency
Isolated Prolongation Of APTT

- **H/O bleeding**
  - Mixing study (repeat APTT with 50:50 mix of patient's plasma and normal plasma)
    - APTT correction > 50%
      - Deficiency of FVIII, FIX, FXI
        - Assay of FVIII, FIX, FXI
    - Poor correction
      - Factor VIII inhibitor
      - Lupus anticoagulant

- **No H/O bleeding**
  - Deficiency of FXIII, HWMK or prekallikrein
THROMBIN TIME (TT)

• Normal range: 8 to 12 seconds
• Prolonged in 1) Afibrinogenaemia
  2) Presence of heparin in plasma
  3) Chronic liver disease
  4) Fibrinogen/fibrin degradation products
FIBRINOlytic Pathway

Prothrombin → Thrombin

Fibrinogen → Fibrin

Thrombin → Thrombus

Fibrin monomer → Fibrin network

Fibrin network → Break down

Plasminogen → Plasmin

Plasmin → FDP

Inhibitors → FDP

Polymerization
SPECIFIC TESTS
SPECIFIC TESTS FOR PRIMARY HAEMOSTASIS

- Platelet aggregation studies
- Flow cytometric detection of glycoprotein on platelet surface
SPECIFIC TEST FOR COAGULATION PHASE

- Mixing studies
- Coagulation factor assays
- Thromboplastin generation test
- Quantitative estimation of fibrinogen
TESTS FOR FIBRINOLYSIS

- Detection of fibrinogen/fibrin degradation products (FDPs)
- Detection of D-dimers
In SUMMARY..
<table>
<thead>
<tr>
<th>BT</th>
<th>PLT C.</th>
<th>PT</th>
<th>APTT</th>
<th>COMMON CAUSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>VWD, asprin, storage pool defect</td>
</tr>
<tr>
<td>↑</td>
<td>D</td>
<td>N</td>
<td>N</td>
<td>Secondary drugs, ITP</td>
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<tr>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>N</td>
<td>Oral anicoagulants, vitamin k defiency, def of F VII</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>Heparin, haemophillia A or B, VWD, inhibitors</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>↑</td>
<td>↑</td>
<td>Heparin, liver disease, vit k deficieny, oral anti coagulant, liver disease</td>
</tr>
<tr>
<td>↑</td>
<td>D</td>
<td>↑</td>
<td>↑</td>
<td>DIC, Liver disease</td>
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<td>↑</td>
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<td>DIC, Liver disease</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Mild VWD, vascular disorder, platelet function defect, FXIIIdefiency</td>
</tr>
</tbody>
</table>
REFERENCES

1. Wintrobe`s clinical hematology; 11 edition; 2nd volume; chapter 51; 1512-1528
2. Essentials of haematology; Kwathalkar 2nd edition chapter 13; 382-398
3. Essentials of clinical pathology; Kwathalkar; chapter 29; 288-331
THANK YOU