L’iter diagnostico di laboratorio nelle coagulopatie congenite emorragiche

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Congenital Hemorrhagic Coagulopathies

Aims of Laboratory Investigation

• To establish the causes of bleeding in patients who have shown evidence of abnormal bleeding

• To detect mild defects in asymptomatic patients (pre-surgical screening)
Congenital Hemorrhagic Coagulopathies

Most Important Screening Test

Good Collection of Clinical History
Congenital Hemorrhagic Coagulopathies

Why should clinical history be collected

• Poor sensitivity of screening tests to detect mild defects
• The type of bleeding may provide valuable clue to its etiology
• Some coagulation abnormalities are not associated with clinical bleeding (*FXII, PreKal, HMWK*)
Aims of the Clinical History

How should clinical history be collected

- Type of bleeding
- Location, frequency, duration, severity
- Whether it is spontaneous or post-traumatic
- Whether other family members have the same symptoms
- The age of appearance of the first symptoms
- Whether other diseases are present
- Whether the patient is taking drugs
Main Bleeding Symptoms

- Bleeding from mucous membranes is a typical feature of platelet disorders
- Soft-tissue bleeding is a typical feature of coagulation disorders
- Umbilical cord or delayed bleeding are typical features of factor XIII deficiency
- Simultaneous bleeding from multiple sites suggests an acute, acquired systemic coagulation or fibrinolytic disorders
Laboratory Tests

Should be aimed at investigating

- **Primary Hemostasis**
  - Platelet vessel-wall interaction
- **Coagulation**
  - Thrombin generation
  - Fibrin formation
- **Fibrinolysis**
  - Fibrin degradation
Laboratory Tests

Should be

• Sensitive
• Limited in number
• Easy to do
• Their results clinically-relevant
Two-step Laboratory Investigation

- **First Step (Simple Screening Tests)**
  - To detect most frequent and well established causes of bleeding

- **Second Step (Specific Tests)**
  - To detect less common causes of bleeding due to abnormalities to which the screening tests are insensitive
First Step
Laboratory Tests

First Step

- Bleeding Time (or alternative tests)
- Platelet Count
- Prothrombin Time (PT)
- Activated Partial Thromboplastin Time (APTT)
Bleeding Time

The time (minutes) needed to stop bleeding from a superficial incision of the skin
## Laboratory Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Materials</th>
<th>Sensitive to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding Time</td>
<td>- Automated device</td>
<td>- Thrombocytopenia</td>
</tr>
<tr>
<td></td>
<td>- Sphygmomanometer</td>
<td>- Thrombocytopathy</td>
</tr>
<tr>
<td></td>
<td>- Filter paper</td>
<td></td>
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<tr>
<td></td>
<td>- Stopwatch</td>
<td></td>
</tr>
</tbody>
</table>
Variables affecting the Bleeding Time

- Depth of incision
- Site of incision
- Venous pressure
- End point
- Effect of drugs

The above variables and the complexity of the bleeding time made most labs to abandon this test.

Alternative tests have been proposed, but not yet completely validated

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Laboratory Tests

First Step

- Bleeding Time (or alternative tests)
- Platelet Count
- Prothrombin Time (PT)
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## Laboratory Tests

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</thead>
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<tr>
<td>Platelet count</td>
<td>- Electronic counter</td>
<td>- Thrombocytopenia</td>
</tr>
<tr>
<td></td>
<td>- Contrast-phase microscope</td>
<td>- Thrombocytosis</td>
</tr>
<tr>
<td></td>
<td>- EDTA (or citrated) whole blood</td>
<td></td>
</tr>
</tbody>
</table>
Further Evaluation of Primary Hemostasis

• **Low Platelet Count**
  - Investigation of thrombocytopenia

• **Prolonged Bleeding Time**
  - Measurement of plasma von Willebrand factor
  - Platelet aggregation studies
First Step

• Bleeding Time (or alternative tests)
• Platelet Count
• Prothrombin Time (PT)
• Activated Partial Thromboplastin Time (APTT)
# Laboratory Tests

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<tbody>
<tr>
<td>PT</td>
<td>- Thromboplastin</td>
<td>- FVII</td>
</tr>
<tr>
<td></td>
<td>- Calcium Chloride</td>
<td>- FX, FV, FII, FI</td>
</tr>
<tr>
<td></td>
<td>- Platelet Poor Plasma</td>
<td>- Oral anticoagulants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Unfractionated heparin (?)</td>
</tr>
</tbody>
</table>
Variables affecting PT

- Type of Thromboplastin
- Citrate concentration \((105-109 \text{ mM})\)
- Calcium Chloride concentration
- Temperature
- Coagulometer
Origin of Thromboplastins

Tissue Factor & Phospholipids

• Human
  - Placenta, recombinant relipidated tissue factor
• Rabbit, Bovine
  - Brain
Prothrombin Time (PT)

Results expression

- Time (seconds)
- % Activity
- Ratio (PTpatient/PTnormal)
- INR (International Normalized Ratio)
Usefulness of the Prothrombin Time (PT)

- Diagnosis and management of
  - Congenital hemorrhagic coagulopathies
  - Disseminated intravascular coagulation
- Prognosis of liver cirrhosis
  - Model of end stage liver disease (MELD)
- Dose-adjustment of VKA
Laboratory Tests

First Step

• Bleeding Time (or alternative tests)
• Platelet Count
• Prothrombin Time (PT)
• Activated Partial Thromboplastin Time (APTT)
# Laboratory Tests

### APTT

<table>
<thead>
<tr>
<th>Test</th>
<th>Materials</th>
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</tr>
</thead>
<tbody>
<tr>
<td>APTT</td>
<td>- Activator</td>
<td>- PK, FXII, HMWK, FXI</td>
</tr>
<tr>
<td></td>
<td>- Phospholipids</td>
<td>- FIX, FVIII</td>
</tr>
<tr>
<td></td>
<td>- Calcium Chloride</td>
<td>- FX, FV, FII, FI</td>
</tr>
<tr>
<td></td>
<td>- Platelet Poor Plasma</td>
<td>- Oral anticoagulants</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Unfractionated heparin, <em>LMWH</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Lupus Anticoagulants</td>
</tr>
</tbody>
</table>
Variables affecting APTT

- Type/Concentration of Activator
- Type/Concentration of Phospholipids
- Activation time
- Citrate concentration \((105-109 \text{ mM})\)
- Calcium chloride concentration
- Temperature
- Coagulometer
Types of APTT Activators

- **Particulate**
  - Kaolin (sensitive, but unpractical)
  - Silica (sensitive and practical)

- **Soluble**
  - Ellagic acid (practical, but rather insensitive)
APTT result expression

- Clotting time (seconds)
- Ratio (patient-to-normal clotting time)
Further Evaluation of Coagulation

PT/APTT Prolongation

Mixing

Correction

Factor assay

Search for Lupus Anticoagulants

No correction

Inhibitor assay
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**Fibrin** (urea soluble) + **Fibrin** (cross linked)

**Factor XIII**

**Common pathway**
- Factor X
- Factor V
- Factor II
- Factor I

**Intrinsic pathway**
- HMWK
- Prekallikrein
- Factor XII
- Factor XI
- Factor IX
- Factor VIII

**Extrinsic pathway**
- Thromboplastin
- Factor VII

**Activated partial thromboplastin time**

**Prothrombin time**

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Need for differential diagnosis when APTT is prolonged owing to coag factors deficiency

• FIX, FVIII or FXI deficiency
  - Hemorrhagic risk
• FXII, Pre-kallicrein or HMWK deficiency
  - No hemorrhagic risk
Usefulness of the APTT

- Diagnosis and management of
  - Congenital hemorrhagic coagulopathies
  - Disseminated intravascular coagulation
- Dose-adjustment of unfractionated heparin therapy
  - Therapeutic interval: 1.5-2.5 times prolongation over the baseline value
- Search for circulating anticoagulants
Second Step

Clinical history of bleeding, but normal first step laboratory tests
Laboratory Tests

Second Step

- **FXIII**
- Platelet Factor 3 (phosphatidylserine)
- Hyperfibrinolysis
- Von Willebrand Factor
- Dysfibrinogenemia
Factor XIII Assay

- Clot solubility in 5M urea
- Functional
- Immunochemical
Laboratory Tests

Second Step

- FXIII
- Platelet Factor 3 (phosphatidylserine)
- Hyperfibrinolysis
- Von Willebrand Factor
- Dysfibrinogenemia
Isolated Defect of PF3 Procoagulant Activity  
*(Scott syndrome)*

- Weiss HJ et al  
  Isolated deficiency of platelet procoagulant activity  

- Toti F et al  
  Scott syndrome, characterized by impaired transmembrane migration of procoagulant phosphatidylserine and hemorrhagic complications, is an inherited disorder  
  *Blood* 1996; 87: 1409

- Charles L. Percy CL et al  
  Laboratory monitoring of Scott Syndrome  
  *Br J Haematol* 2009; 149: 803
Laboratory Tests

Second Step

- FXIII
- Platelet Factor 3 (phosphatidylserine)
- Hyperfibrinolysis
- Von Willebrand Factor
- Dysfibrinogenemia
Fibrinolysis

Histidin-rich Glycoprotein

TAFI

Tissue Plasminogen Activator
Activator
Inhibitor

Plasminogen

Intrinsic activation (FXIIa, Kal, etc.)

Tissue Plasminogen Activator

Urokinase Pro-Urokinase

Plasmin

Plasmin inhibitor

Fibrin

Fibrin degradation
Congenital Deficiency of Plasmin Inhibitor

- **Homozygotes**
  - Severe hemophilia-like bleeding tendency since childhood
  - Rebleeding from wounds
- **Heterozygotes**
  - About 20% of patients present with mild bleeding tendency (easy bruising, oozing from dental extractions)
Laboratory Tests

Second Step

- FXIII
- Platelet Factor 3 (phosphatidylserine)
- Hyperfibrinolysis
- Von Willebrand Factor
- Dysfibrinogenemia
The APTT & Willebrand disease

Mild forms of Willebrand disease may present with (near) normal APTT
Laboratory Tests

Second Step

• FXIII
• Platelet Factor 3 (phosphatidylserine)
• Hyperfibrinolysis
• Von Willebrand Factor
• Dysfibrinogenemia
Dysfibrinogenemia

Main Characteristics

- Abnormal fibrinogen in plasma
- Low functional fibrinogen (Clauss method)
- Normal or high immunochemical fibrinogen
- Prolonged thrombin clotting time
Dysfibrinogenemia

Symptoms

- None
- Hemorrhage
- Arterial and/or Venous Thromboembolism
Conclusions

• A rational two-step approach combining
  - Clinical data
  - Laboratory data
• Helps identifying the majority of congenital hemorrhagic coagulopathies