12th Annual Canadian Blood Services International Symposium

Plasma: Transfuse it, Fractionate it or Forget it?

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Plasma Testing in the Clinical Coagulation Laboratory: New drugs, new problems.

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What prevents us from bleeding?
Series of inter-related processes'...

- **Primary hemostasis**
  - Involves the interaction of von Willebrand factor and platelets

- **Secondary hemostasis**
  - End result: prothrombin is activated to thrombin, converting fibrinogen to fibrin and the formation of a fibrin clot

- **Clot stabilization**
  - Activation of factor XIII by thrombin, in the presence of fibrin

- **Wound healing**
  - Delayed activation of the fibrinolytic system
Introduction

- Laboratory tests are essential for diagnosing bleeding disorders.
- Congenital or acquired abnormalities can be found in any part of the hemostasis pathway.
- Coagulation screening tests are commonly performed as the initial investigation of a bleeding disorder.
<table>
<thead>
<tr>
<th>Cause and pattern of abnormalities</th>
<th>PT/INR</th>
<th>APTT</th>
<th>TT</th>
<th>fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen deficiency (hypofibrinogenemia) or dysfunction (dysfibrinogenemia)</td>
<td>N – ↑</td>
<td>N – ↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Afibrinogenemia</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>ND</td>
</tr>
<tr>
<td>FVII deficiency</td>
<td>↑</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>FVIII, FIX, and/or FXI deficiency</td>
<td>N</td>
<td>↑ †</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Acquired or congenital hemophilia, with an inhibitor</td>
<td>↑</td>
<td>↑ †</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>FII, FV, and/or FX deficiency</td>
<td>↑</td>
<td>↑ †</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Factor deficiencies not associated with bleeding (FXII, high molecular weight kininogen or prekallikrein deficiency)</td>
<td>N</td>
<td>↑ †</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
<td>N – ↑</td>
<td>N – ↑ †</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Lupus anticoagulant with FII deficiency</td>
<td>↑</td>
<td>↑ †</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Unfractionated heparin - therapy or sample contamination</td>
<td>N – ↑</td>
<td>↑</td>
<td>↑ †*</td>
<td>N</td>
</tr>
<tr>
<td>Low molecular weight heparin therapy</td>
<td>N</td>
<td>N – ↑</td>
<td>N</td>
<td>↑ †</td>
</tr>
<tr>
<td>Direct thrombin inhibitors</td>
<td>N – ↑</td>
<td>N – ↑</td>
<td>↑ †</td>
<td>N</td>
</tr>
<tr>
<td>Direct inhibitors of FXa</td>
<td>N – ↑</td>
<td>N – ↑</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Liver disease† (if early, often affects FVII, FIXI and/or FXII; if late or end stage, fibrinogen is usually low; spares FVIII but can affect all other factors)</td>
<td>N – ↑</td>
<td>N – ↑</td>
<td>N</td>
<td>↓ – N – ↑</td>
</tr>
<tr>
<td>Vitamin K deficiency (or treatment with a vitamin K antagonist) which reduce levels of FVII and also FII, FIXI and FX†</td>
<td>↑</td>
<td>N – ↑</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Fibrinolytic therapy</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Consumptive coagulopathy†</td>
<td>N – ↑</td>
<td>↑</td>
<td>N</td>
<td>N – ↓</td>
</tr>
<tr>
<td>Dilutional coagulopathy†</td>
<td>N – ↑</td>
<td>N – ↑</td>
<td>N</td>
<td>↓ – N – ↑</td>
</tr>
<tr>
<td>VWD</td>
<td>N</td>
<td>N – ↑</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Preanalytical error – collected in potassium EDTA§</td>
<td>↑</td>
<td>↑</td>
<td>N</td>
<td>↑</td>
</tr>
<tr>
<td>Preanalytical error – serum instead of plasma</td>
<td>NC</td>
<td>NC</td>
<td>NC</td>
<td>ND</td>
</tr>
</tbody>
</table>

†, elevated levels; ↓, reduced levels; N, normal; NC, no clot; ND, not detectable.
<table>
<thead>
<tr>
<th>Test</th>
<th>Estimated Sensitivity (%)</th>
<th>Estimated Specificity (%)</th>
<th>Purpose of test and defects detected</th>
<th>Abnormalities that don’t cause bleeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT</td>
<td>2.1</td>
<td>98</td>
<td>Congenital or acquired deficiencies of intrinsic (PK, HMWK, XII, XI, IX, VIII) and common pathway (FX, FV, FII).</td>
<td>Contact factors deficiencies (FXII, PK, HMWK) and most lupus anticoagulants.</td>
</tr>
<tr>
<td>PT/INR</td>
<td>1.0</td>
<td>&gt;99</td>
<td>Congenital or acquired deficiencies of FVII and common pathway.</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>1.0</td>
<td>96</td>
<td>Congenital and acquired defects in fibrinogen and fibrin polymerization, ↑ FDPs.</td>
<td>Valproic acid therapy and some paraproteins can prolong test.</td>
</tr>
<tr>
<td>Clauss Fibrinogen</td>
<td>1.0</td>
<td>&gt;99</td>
<td>Quantitative and qualitative defects in fibrinogen.</td>
<td></td>
</tr>
</tbody>
</table>

Table modified from: Hayward and Moffat. Int J Lab Hematol. 35:322-33; 2013.
Case # 1

- A 36 year old female was referred to the High Risk Clinic at an acute care hospital.
- After extensive bleeding with the loss of her 3rd pregnancy she was admitted to hospital.
- On admission:
  - PT/INR 2.3, RI 0.8 – 1.2 INR
  - APTT 44, RI 22 – 35 s
  - TT 108, RI 20 – 30 s
  - Fibrinogen 0.70, RI 1.6 – 4.2 g/L
Case # 1
Additional laboratory testing

- Urea clot (FXIII) solubility test: Normal
- Factor XIII activity level:
  0.79 U/mL       RI  0.60 – 1.69 U/mL

- Immunological fibrinogen (2 samples)
  0.60 g/L       RI  1.6– 4.2 g/L
  0.80 g/L
Immunological Fibrinogen

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</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>1 = 1/10 = 0.60 g/L</td>
<td>2 = 1/20 = 0.65 g/L</td>
<td>3 = 1/40 = 0.65 g/L</td>
<td>4 = 1/5 = 0.60 g/L</td>
</tr>
</tbody>
</table>

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At the time, the patient was treated with Haemocomplettan® P
  ◦ Plasma derived fibrinogen concentrate
  ◦ Requires SAP

Currently RiaSTAP™ available
  ◦ Also a plasma derived fibrinogen concentrate
  ◦ Does not require SAP
New Oral Anticoagulants

Examples of:
- Direct factor Xa inhibitors
  - Rivaroxaban
  - Apixaban
- Indirect factor Xa inhibitors
  - Fondaparinux
  - Idraparinux
  - Oral heparins
- Factor IIa inhibitors
  - Hirudin
  - Bivalirudin
  - Argatroban
  - Dabigatran
  - Orally available heparins
PT and APTT - Dabigatran

- PT reagents vary in sensitivity
- PT -13% to 43% results normal at therapeutic drug levels
- APTT prolongation is curvilinear with less reagent variation
- 11% to 26% results normal at therapeutic drug levels
PT and APTT – Rivaroxaban

- PT and APTT – sensitivity varies between reagents
- PT - 10% to 52% results normal at therapeutic drug levels
- APTT - 31% to 59% results normal at therapeutic drug levels
PT and APTT - Apixaban

- PT is generally normal at therapeutic drug concentrations
  - Exception: Triniclot PT Excel S reagent
- APTT prolongation is curvilinear with a concentration dependent prolongation that plateaus at 200 ng/mL
Clauss Fibrinogen - Dabigatran

Legend
Green: Fibri-Prest (Stago)
Blue: Dade Thrombin (Siemens)
Yellow: Multifibren U (Siemens)
Red: Fibrinogen C (IL)
Case # 2

- 73 year old male, retired teacher
- Right thigh bleed on warfarin
- One year later presented with left leg pain
  - Received two doses of fragmin for suspected calf vein thrombosis
  - Within 24 hours, leg became grossly swollen and discoloured due to hemorrhage
  - Hemoglobin 60 g/L, transfused 3 units red cells
Case # 2

- Lab testing initiated from community hospital
  - PT, APTT, fibrinogen, VWF levels: All normal
  - Factor assays: All normal

- Further specialized laboratory studies
  - Urea clot solubility test: Deficient factor XIII activity
    - No clot after 24 hours at room temperature
  - FXIII antigen assays:
    - FXIII subunit A: <0.05 U/mL  RI: 0.67-1.39 U/mL
    - FXIII subunit B: 0.48 U/mL  RI: 0.73-1.17 U/mL
Factor XIII

- Plasma FXIII is a transglutaminase
- Consists of:
  - 2 - subunit A
    - Catalytic subunit
    - Calcium binding site
  - 2 - subunit B (formerly subunit S)
    - Carrier subunit
- Zymogen activated by thrombin and calcium, in the presence of fibrin.
Factor XIII Function

- Stabilizes initial fibrin clot
  - Forms covalent bonds which
    - cross-link fibrin monomers
    - cross-links $\alpha_2$-antiplasmin into fibrin clot
Factor XIII Deficiency – Why is the PT and APTT normal?

- Thrombin cleaves FPA and FPB from fibrinogen to form fibrin monomers.
- Monomers polymerize to form soluble fibrin.
- FXIII is activated and forms insoluble fibrin.
- In-vitro clot detection systems detect soluble (non-cross linked) fibrin.
Factor XIII Clot Solubility Assays

- Qualitative screening test
- abnormal when factor XIII is reduced to \( \leq 0.02 - 0.03 \text{ U/mL} \)
- Principle:
  - Non-crosslinked fibrin can be dissolved by adding denaturing agents (i.e. 5 M urea, 2% monochloroacetic acid) whereas cross-linked fibrin do not dissolve.
Normal Factor XIII  No Factor XIII
FXIII - Sensitivity and Specificity

- Not reported
- Clot solubility assays only detect severe deficiencies
- FXIII activity assays are useful for monitoring therapy
  - Able to quantitate a wider range of levels
- Most congenital deficiencies are severe subunit A deficiencies

Back to Case #2

- Patient was treated with Fibrogammin® P
  - human plasma derived FXIII product

- Regular laboratory monitoring of patient’s samples
  - FXIII clot solubility test
  - FXIII activity levels
Conclusions

- **PT and APTT:**
  - Screen for numerous coagulopathies.
  - Demonstrate excellent specificity but poor sensitivity.
  - Not suitable for monitoring new oral anticoagulants due to the wide variability in sensitivity between reagents.
  - normal with a factor XIII deficiency, requiring specialized testing.

- Dabigatran may cause false low Clauss fibrinogen levels, dependent on thrombin reagent used.
Thank you for your attention.