Improving Acute Care with Coagulation Mixing Studies

George A Fritsma MS, MLS
The Fritsma Factor,
Your interactive Hemostasis Resource℠
Sponsored by Precision BioLogic
Dartmouth, Nova Scotia
george@fritsmafactor.com
www.fritsmafactor.com

Coagulation Mixing Studies
Learning Objectives

At the conclusion of this webinar, the participant...
1. Prepares a stepwise PTT and PT mixing study protocol
2. Indicates the clinical purposes for PTT mixing studies
3. Explains why the mixing study is an acute care assay
4. Correlates mixing study results with coagulation test results

Mixing Study
A first-line investigation to differentiate a coagulation deficiency from an inhibitor


Case: 32-yr Female
Pre-op Screen
Six weeks post-partum
Easy bruising, frequent nosebleeds, menorrhagia

Pre-op Screen
32-yr Female, 6 Weeks Post-partum

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patient</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>11.8 g/dL</td>
<td>12–15 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>12.4 s</td>
<td>9.8–12.6 s</td>
</tr>
<tr>
<td>PTT (APTT)</td>
<td>42.5 s</td>
<td>25–35 s</td>
</tr>
<tr>
<td>PLT count</td>
<td>310,000/µL</td>
<td>250–450,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>320 mg/dL</td>
<td>220–498 mg/dL</td>
</tr>
</tbody>
</table>

Isolated, prolonged PTT response? 1:1 PTT mix

Rule Out Heparin, Dabigatran

<table>
<thead>
<tr>
<th>Assay</th>
<th>Patient</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>14 s</td>
<td>&lt;21 s</td>
</tr>
</tbody>
</table>

- R/O dabigatran and unfractionated heparin (UFH)
- Outpatient—consider dabigatran
- Inpatient—unrecorded UFH flush of vascular catheter
- If dabigatran, discontinue, cancel order
- If UFH, use Hepsoor (polybrene) or Hepzyme, proceed
- If no UFH, perform 1:1 PTT mix to differentiate factor deficiency from factor-specific inhibitor or “non-specific inhibitor” lupus anticoagulant (LA)
**PTT Mixing Study: Cheap and Basic**

- Start within 2 h to avoid specimen degradation
- Factors V (FV) and VIII (FVIII) are labile
- Platelet factors (mostly FV) released to plasma
- Ensure pt plasma is platelet-poor (free), <10,000/uL
- Mix plasma 1:1 with pooled normal plasma (NP) and perform immediate PTT on mixture
- PTT of 1:1 mix corrects to ≤10% longer than NP PTT
- No correction: 1:1 mix is >10% longer than NP PTT
- Non-specific inhibitor, usually LA
- Specific inhibitor (anti-FVIII) may be present, usually requires 37°C incubation

**PTT Mixing Study Using 10% Rule**

- 100 uL
- PTT reagent
- PTT ≤33 s: Correction
- >33 s: No correction

**1:1 PTT Mix with Incubation**

- PTT of immediate mix ≤10% longer than NP
  - Correction: factor deficiency? But first…
  - Incubate 1:1 mix, 37°C, 1–2 h and repeat
- Correction after 37°C mix = factor deficiency
- Incubated PTT remains >10% longer than NP
  - Specific inhibitor such as anti-FVIII
    - IgG: Temp dependent, may require incubation
    - However, some FVIII neutralization within 10 m
    - May detect in immediate mix

**1:1 PTT Mix After 37°C Incubation**

- Only when unincubated mix corrects
- Must also incubate normal control plasma
- Compare mix PTT to incubated NP PTT
- May also detect temp-dependent LA
  - ~15% of LAs are temp-dependent

Mixing Study Result
32-yo Female, 6 Weeks Post-partum

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>RI</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT</td>
<td>42.5 s</td>
<td>25–35 s</td>
<td>Confirms previous PTT</td>
</tr>
<tr>
<td>PTT/control 1:1 mix</td>
<td>32.1 s</td>
<td>Control 30 s</td>
<td>Commercial platelet-free control plasma (NP)</td>
</tr>
<tr>
<td>PTT/control 1:1 mix</td>
<td>37.3 s</td>
<td>Control 35 s</td>
<td>Incubate both 1:1 mix and NP</td>
</tr>
</tbody>
</table>

Conclusion: immediate and incubated mix PTTs correct, suspect factor deficiency, arrange for factor assays and von Willebrand disease workup.

Factor Assay Results
32-yo Female, 6 Weeks Post-partum

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>RI</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII</td>
<td>39%</td>
<td>50–150%</td>
<td>VWD?</td>
</tr>
<tr>
<td>Factor IX</td>
<td>92%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor XI</td>
<td>131%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor XII</td>
<td>113%</td>
<td></td>
<td>XII, HMWK &amp; PK deficiency not associated with bleeding</td>
</tr>
<tr>
<td>HMWK</td>
<td>ND</td>
<td>65–135%</td>
<td></td>
</tr>
<tr>
<td>PK</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PT and PTT Test Results in Inherited Coagulopathies

<table>
<thead>
<tr>
<th>PT</th>
<th>PTT</th>
<th>Single Factor Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>Normal</td>
<td>VII</td>
</tr>
<tr>
<td>Long</td>
<td>Long</td>
<td>X, V, II, and fibrinogen¹</td>
</tr>
<tr>
<td>Normal</td>
<td>Long</td>
<td>VIII, IX, XI</td>
</tr>
</tbody>
</table>

PTT Mix: Why Does This Work?
- Hypothetical 20% F VIII level prolongs PTT
- PTT rgt calibrates to prolong at 30–40% FVIII, IX, XI
- Add NP with established 100% factor level
- 1:1 mix, average of 100% and 20% = 60% (corrects)
- Hypothetical anti-FVIII or lupus anticoagulant
- With typical avidity, retains ability to prolong the mix

Case
52-yo Athletic Female

Pre-op screen for total hip replacement
Isolated Prolonged PTT: Differential

- Could be nothing: 5% of normals exceed limit
- Preanalytical variable: green or lavender-closure tube, hemolysis, lipemia, clotted specimen
- Outpatient: dabigatran
- Inpatient: unreported UFH
- Congenital single factor deficiency: VIII, IX, or XI, hemophilia A, B, or C with bleeding, VWD
- Congenital FXII, PK, or HMWK without bleeding
- FVIII inhibitor (acquired hemophilia) with bleeding
- Lupus anticoagulant (LA)

Patient reports no bleeding or bruising, no thrombosis

### 52-yo Athletic Female

**Screen Prior to Hip Replacement Surgery**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>14.1 g/dL</td>
<td>12–15 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>11.2 s</td>
<td>9.8–12.6 s</td>
</tr>
<tr>
<td>PTT</td>
<td>58 s</td>
<td>25–35 s</td>
</tr>
<tr>
<td>PLT</td>
<td>170,000/µL</td>
<td>150–400,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>410 mg/dL</td>
<td>220–498 mg/dL</td>
</tr>
</tbody>
</table>

Patient reports no bleeding or bruising, no thrombosis

### 52-yo Female PTT Mixing Study

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>17 s</td>
<td>RI: &lt; 21 s, rules out dabigatran</td>
</tr>
<tr>
<td>PTT</td>
<td>58 s</td>
<td>RI: 25–35 s</td>
</tr>
<tr>
<td>PTT NP</td>
<td>28 s</td>
<td>Correction if ≤ 30.8 s (10%)</td>
</tr>
<tr>
<td>1:1 mix</td>
<td>35 s</td>
<td>25% over NP = no correction</td>
</tr>
</tbody>
</table>

What is the next step?

### Acute Care Mixing Study Algorithm

1. **Isolated prolonged PTT**
   - **TT long**
   - Heparinase or polybrene
   - Pt & NP 1:1 mix
   - Correction
   - Incubated 37°C
   - Pt & NP 1:1 mix
   - No correction
   - Factor assay
   - No correction

2. **Initial PTT 48 s, RI 25–35; 1:1 mix prolongs to 54 s**
   - LA "cofactor" effect may be prothrombin binds LA
   - Or maybe LA potentiates clotting via annexin V?
   - Mix reverses potentiation?

### Mixing Study Considerations

- **Preanalytical variables**
  - Anti-Xa rivaroxaban, apixaban, edoxaban prolong PT, PTT
  - Dabigatran and UFH prolong PTT
  - Clotted, hemolyzed, lipemic specimen
  - Underfilled tube, wrong anticoagulant
  - PT & NP must be platelet-poor (free), <10,000/uL
  - Clg at 2500 g/10 m or double-spin
  - Heparinase/polybrene neutralize ≤ 1 unit/mL UFH
  - Anti-FVIIIs may generate immediate neutralization
  - Weak LAs may be missed in 1:1 mix; ask for consult
  - Select a more LA-sensitive PTT reagent or request 4:1 mix
  - Most LA require incubation

- **Mostly Not so much**

### The “LA Cofactor Effect”

- Initial PTT 48 s, RI 25–35; 1:1 mix prolongs to 54 s
- LA "cofactor" effect may be prothrombin binds LA
- Or maybe LA potentiates clotting via annexin V?
- Mix reverses potentiation?

Normal Plasma Source?
- Home brew: ~20 normal plasmas, male ≠ female
  - Ensure plasma is platelet-poor; < 10,000/uL
  - Ensure NP has ~100% of all factors; PTT ≅ MRI
  - For instance, elevated FVIII causes false negatives
  - Screen for LA, specific factor inhibitors, HBV, HCV, HIV
  - Aliquot and freeze
- Or purchase commercial plasma
  - GMP meets all criteria
  - Frozen meets all criteria
  - Lyophilized acceptable when validated in house
- Processed with stabilizers


What Limit Defines Correction?
- No Consensus; Fritsma Factor Quick Question Results
- Limit based on fixed PTT value from reference interval
  - 1:1 mix within RI upper limit (95% or 99% CI, 39%)
  - 1:1 mix within RI upper limit + 5 seconds (8%)
  - 1:1 mix within mean of RI + 2 or 3 SD (0%)
- Limits based on NP PTT value
  - 1:1 mix within NP PTT value + 5 seconds (14%)
  - 1:1 mix within NP PTT + 10% (32%)
- Limit formula using patient, NP, and 1:1 mix
  - Must incubate patient sample, NP, and 1:1 mix
  - Chang’s % deviation; Rosner index
- Combo of RI and Rosner (dedicated RI for mix, 7%)

Chang Formula Based on % Correction
\[
\text{% Correction} = \frac{\text{Patient PTT} - \text{1:1 mix PTT}}{\text{Patient PTT} - \text{NP PTT}} \times 100
\]

Factor Deficiency = ≥ 75%
Inhibitor = < 75%
% Correction Chang formula verified by local laboratory

Rosner Index Based on Ratio
\[
\text{Rosner Index} = \frac{\text{1:1 mix PTT} - \text{NP PTT}}{\text{Patient PTT}} \times 100
\]

Inhibitor ≥ 11
Correction < 11
Rosner index limit validated by local laboratory

59-yo Male Former Hockey Player
Screen Prior to Knee Replacement Surgery

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>14.8 g/dL</td>
<td>12–15 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>11.2 s</td>
<td>9.8–12.6 s</td>
</tr>
<tr>
<td>PTT</td>
<td>38 s</td>
<td>25–35 s</td>
</tr>
<tr>
<td>PLT</td>
<td>310,000/µL</td>
<td>150–400,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>390 mg/dL</td>
<td>220–498 mg/dL</td>
</tr>
</tbody>
</table>

Patient reports no bleeding or bruising, no thrombosis
**When to Perform Mixing Study**

- Any PTT > RI upper limit
- Any PTT > RI upper limit + 5 seconds
- Any PTT > RI upper limit with consult
- Is patient bleeding or clotting?
- Possible ‘weak’ LA: use 4:1 mix
- Lupus sensitive PTT reagent
- Factor sensitive PTT reagent

---

**Some Practical Considerations**

- If you use a value slightly longer than the RI limit and define correction as return to the RI you miss most inhibitors.
- If you perform mixing studies on prolonged PTTs from inpatients, at least 50% will be due to anticoagulant therapy.
- If you call the unit on any prolonged PTT you are likely to get no information.

---

**59-yr Male Former Hockey Player**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>17 s</td>
<td>RI: &lt; 21 s, rules out dabigatran</td>
</tr>
<tr>
<td>PTT</td>
<td>38 s</td>
<td>RI: 25–35 s</td>
</tr>
<tr>
<td>PTT NP</td>
<td>31 s</td>
<td>Correction if &lt; 34.1 s (10%)</td>
</tr>
<tr>
<td>1:1 mix</td>
<td>35 s</td>
<td>Correction? No correction?</td>
</tr>
</tbody>
</table>

What is the next step?

---

**2-yr Hemophilic Boy**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>11.8 g/dL</td>
<td>9.6–15.6 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>11.2 s</td>
<td>9.8–12.6 s</td>
</tr>
<tr>
<td>PTT</td>
<td>65 s</td>
<td>25–35 s</td>
</tr>
<tr>
<td>PLT</td>
<td>310,000/µL</td>
<td>150–400,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>390 mg/dL</td>
<td>220–498 mg/dL</td>
</tr>
</tbody>
</table>

Inflamed, swollen knee and ankle
Mixing Study Result

2-yo Hemophilic Boy

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>RI</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT</td>
<td>65 s</td>
<td>25–35 s</td>
<td>Confirms previous PTT</td>
</tr>
<tr>
<td>PTT/control 1:1 mix immediate</td>
<td>33.5 s</td>
<td>Control 30 s</td>
<td>Correction</td>
</tr>
<tr>
<td>PTT/control 1:1 mix 1 h at 37°C</td>
<td>47.9 s</td>
<td>Control 35 s</td>
<td>Control is incubated alone and with mix</td>
</tr>
</tbody>
</table>

Conclusion: Anti-FVIII inhibitor

Factor VIII Assay

- Dilute plasma 1:10, add FVIII-depleted rgt plasma 1:1
- Add PTT reagent, incubate 3 minutes
- Add CaCl₂, record interval to clot formation
- Compare result in seconds to dilution formation

Factor VIII Assay Dilutions Parallelism Indicates No Inhibitor

<table>
<thead>
<tr>
<th>Plasma Dilution</th>
<th>Seconds</th>
<th>Raw Factor VIII Activity</th>
<th>Computed Factor VIII Activity (× dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 &quot;undiluted&quot;</td>
<td>90 s</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>1:20</td>
<td>104 s</td>
<td>10%</td>
<td>20% (parallel)*</td>
</tr>
<tr>
<td>1:40</td>
<td>107 s</td>
<td>5%</td>
<td>20% (parallel)</td>
</tr>
<tr>
<td>1:80</td>
<td>110 s</td>
<td>2.5%</td>
<td>20% (parallel)</td>
</tr>
</tbody>
</table>

* <10% difference from undiluted indicates parallelism, no inhibitor

55-YO Male with Atrial Fibrillation

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB</td>
<td>13.8 g/dL</td>
<td>12–15 g/dL</td>
</tr>
<tr>
<td>PT</td>
<td>17.2 s</td>
<td>9.8–12.6 s</td>
</tr>
<tr>
<td>PTT</td>
<td>159 s</td>
<td>25–35 s</td>
</tr>
<tr>
<td>PLT</td>
<td>310,000/µL</td>
<td>150–400,000/µL</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>20 mg/dL</td>
<td>220–498 mg/dL</td>
</tr>
</tbody>
</table>

References:
55-yo Male with Atrial Fibrillation

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
<th>RI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT</td>
<td>159 s</td>
<td>25–35 s</td>
</tr>
<tr>
<td>TT</td>
<td>&gt; 150 s</td>
<td>&lt; 21 s</td>
</tr>
<tr>
<td>PTT/control 1:1 mix immediate</td>
<td>78 s</td>
<td>Control 30 s</td>
</tr>
<tr>
<td>PT/control 1:1 mix immediate</td>
<td>15.2 s</td>
<td>Control 12 s</td>
</tr>
</tbody>
</table>

What do you recommend?

If the PT is Prolonged

- Congenital deficiencies of II, V, VII, or X
- PT and PTT long: II, V, X
- PT only: VII, skip mixing and go to factor assay
- Prevalence: 500,000–1,200,000
- Liver disease: PT prolongs before PTT due to des-carboxy II, VII, and X, reduced factor V
- Vit K deficiency: des-carboxy II, VII, and X
- Anti-Xa direct oral anticoagulants
  - Rivaroxaban, apixaban, edoxaban

Isolated Prolonged PTT: Summary

- Random benign prolongation, 95% CI
- Lupus anticoagulant: 1–3%
  - Drug reaction producing transient LA
- Unrecorded heparin, dabigatran, oral anti-Xa
- Known hemophilic who fails FVIII concentrate Rx
- Hemorrhage or ecchymoses signal acquired coagulopathy; vitamin K deficiency, liver disease
- Specific inhibitor, anti-FVIII: post partum, malignancy, autoimmune disorders, > 60 YO

Develop Mixing Study Reliability

- Test PTT reagent sensitivities
  - 30–40% FVIII, FIX, FXI
- Select Intermediate sensitivity to LA
- NP consistency: ~100% activity for all factors
- Consultation for equivocal patient results
- Employ consistent correction limit

Perform Mixing Studies Locally

- Unexpected isolated prolonged PTT or PT requires immediate action
- Delay results in specimen deterioration
- Perform locally, results may immediately direct therapy
- Forward results to ref lab to direct follow-up
Thanks for listening!

Questions?