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
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
Case: 32-yo Female
Pre-op Screen

Six weeks post-partum
Easy bruising, frequent nosebleeds, menorrhagia
### Pre-op Screen

**32-yo 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>
<tr>
<td><strong>Isolated, prolonged PTT response? 1:1 PTT mix</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Rule Out Heparin, Dabigatran

- 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 Hepsorb (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)

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<tr>
<td>TT</td>
<td>14 s</td>
<td>&lt;21 s</td>
</tr>
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</table>
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
  - Factor deficiency
- 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 37C incubation
PTT Mixing Study

Patient plasma 42.5 s + Normal plasma 30 s = 1:1 mix

Manufacturer’s value confirmed by QA staff
PTT Mixing Study
Using 10% Rule

NP value is 30s

100 uL 1:1 mix

100 uL PTT reagent

100 uL CaCl₂

1:1 mix + PTT rgt

1:1 mix + PTT rgt + CaCl₂

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
    - IgG₄: 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

37C Incubated 1:1 PTT Mix

Patient plasma

PTT 42.5 s

Incubate 1 h, then perform PTT

Incubated PTT 35 s

1:1 mix: incubate 1 h, then perform PTT

PTT of Mix

≤38.5 s: Correction

>38.5 s: No correction

Still using 10% prolongation as correction cutoff
### Mixing Study Result

**32-yo Female, 6 Weeks Post-partum**

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<td>25–35 s</td>
<td>Confirms previous PTT</td>
</tr>
<tr>
<td>PTT/control 1:1 mix immediate</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 1 h at 37°C</td>
<td>37.3 s</td>
<td>Control 35 s</td>
<td>Incubate both 1:1 mix and NP</td>
</tr>
</tbody>
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**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

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<th>RI</th>
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</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>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
PTT rgt: Ca^{++}, activator, phosphatidyl serine; prolonged by XII, PK, HMWK, XI, IX, VIII, X, V, II, Fg deficiency; heparin, DTIs, LA

PT rgt: tissue factor, Ca^{++}, phosphatidyl serine; prolonged by VII, X, V, II, Fg deficiency; direct anti-Xa and coumadin Rx

Figure courtesy of Margaret G. Fritsma, Rodak’s Hematology, 5th Edition, 2015
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 fibrinogen&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal</td>
<td>Long</td>
<td>VIII, IX, XI&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>PT & PTT prolonged when fibrinogen is <100 mg/dL, perform fibrinogen assay
<sup>2</sup>Contact factor deficiencies XII (1–3% prevalence), prekallikrein (PK, Fletcher), or high molecular weight kininogen (HMWK, Fitzgerald) also prolong PTT results, but no bleeding
PTT Mix: Why Does This Work?

- Hypothetical 20% F VIII level prolongs PTT
  - PTT rgts calibrated 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

\[
\text{Patient} \quad \frac{20\%}{20\% \text{ FVIII}} \quad + \quad \text{Normal} \quad \frac{100\%}{100\% \text{ FVIII}} \quad = \quad \text{1:1 Mix: 60\% FVIII}
\]
Case

52- yo Athletic Female

Pre-op screen for total hip replacement
52-yo Athletic Female
Screen Prior to Hip Replacement Surgery

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<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
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)
## 52-yo Female PTT Mixing Study

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<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 $\leq 30.8$ 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

- **Isolated prolonged PTT**
  - **TT long**
  - Heparinase or polybrene
    - If no correction, dabi—stop here
  - **TT**
    - Pt & NP 1:1 mix
      - Correction
        - Incubated 37C Pt & NP 1:1 mix
          - Correction
            - Factor assay
          - No correction
            - LA profile
        - No correction
          - FVIII inhibitor
            - Consider LA
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
  - Cfg at 2500 g/10 m or double-spin

- Heparinase/polybrene neutralize ≤ 1 unit/mL UFH

- Anti-FVIIIIs 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
  - 15% of LAs require incubation
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

\[
\text{% Correction} = \left( \frac{\text{Patient PTT} - \text{1:1 mix PTT}}{\text{Patient PTT} - \text{NP PTT}} \right) \times 100
\]

\[
\begin{align*}
\text{Patient PTT} &- 1:1 \text{ mix PTT} \\
\text{Patient PTT} &- \text{NP PTT}
\end{align*}
\]

\[
\begin{align*}
\text{% Correction} & = \frac{42.5 - 32.1}{42.5 - 30} = \frac{10.4}{12.5} = 0.83 = 83\%
\end{align*}
\]

Factor Deficiency = \geq 75%

Inhibitor = \leq 75%

% Correction Chang formula verified by local laboratory

Rosner Index Based on Ratio

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

Inhibitor \( \geq 11 \)  
Correction \(< 11 \)

Rosner index limit validated by local laboratory

59-yo Male
Former Hockey Player

Total knee replacement preop
**59-yo Male Former Hockey Player Screen Prior to Knee Replacement Surgery**

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</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

When to Perform Mixing Study
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-yo Male Former Hockey Player

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<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?
59-yo Male Former Hockey Player
Clinical Consult

- Consult: if no thrombosis or bleeding, go no further
- Thrombosis: perform mix using 4:1 patient to NP
- Or choose PTT reagent that is LA-sensitive
- If anatomic bleeding symptoms, test FVIII, FIX, FXI
  - Vitamin K deficiency, renal insufficiency, liver disease, malignancy, VWD
2-yr Hemophilic Boy
# 2-yo Hemophilic Boy

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<tr>
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Inflamed, swollen knee and ankle
### Mixing Study Result
#### 2-yo Hemophilic Boy

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<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 CaCl$_2$, record interval to clot formation
- Compare result in seconds to dilution curve
## 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 “undiluted”</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
### FVIII Assay Dilutions

**non-Parallelism Indicates Inhibitor**

<table>
<thead>
<tr>
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<th>Seconds</th>
<th>Raw Factor VIII Activity</th>
<th>Computed Factor VIII Activity (× dilution)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 “undiluted”</td>
<td>80 s</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>1:20</td>
<td>93 s</td>
<td>8%</td>
<td>16%</td>
</tr>
<tr>
<td>1:40</td>
<td>107 s</td>
<td>5%</td>
<td>20%</td>
</tr>
<tr>
<td>1:80</td>
<td>108 s</td>
<td>4%</td>
<td>32%</td>
</tr>
</tbody>
</table>

* >10% difference from undiluted, rising = non-parallel, implies inhibitor

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55-YO Male with Atrial Fibrillation
55-yo Male with Atrial Fibrillation

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<td>9.8–12.6 s</td>
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<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>
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<td>Fibrinogen</td>
<td>20 mg/dL</td>
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55-yo Male with Atrial Fibrillation

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<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: 500,000–1:2,000,000

- 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, edoxabban
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


PrecisionBioLogic
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
The participant…
1. Prepares a stepwise PTT and PT mixing study protocol
2. Indicates the clinical purpose for PTT mixing studies
3. Explains why the mixing study is an acute care assay
4. Correlates mixing study results with coagulation test results
Thanks for listening!

Questions?