Fallacies of Coagulation Testing

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Disclosure

Dr. Sarode has nothing to disclose.
Routine Coagulation Tests

- PT and PTT
- Fibrinogen and D-Dimers
  - PT and PTT have diagnostic value in patients with bleeding disorders
  - They have not been shown to assess bleeding risk in a non-bleeding patient.
Partial Thromboplastin Time (PTT)

- XII
- XI
- IX
- VIII

Intrinsic

Prothrombin Time (PT)

- TF
- VII

Extrinsic (TFP)

Common

Fibrinogen → Fibrin clot

X, V, II
Prothrombin Time

Pt Plasma

Tissue Thromboplastin and Ca++

Clotting time 9 - 12.5"

Sources
Brains: Human, Rabbit, Goat
Placenta: Human
Recombinant
Partial Thromboplastin Time

Plasma

Contact activator

Kaolin
Ellagic acid

Phospholipid

CaCl$_2$

LA

Clotting Time $>35''$
Case 1

- A 4 year old WB seen by a Pediatrician in his office for severe cough and fever for last 4 days. The child had enlarged tonsils. He has had similar bouts several times last year. The ENT surgeon wanted to remove tonsils. This time pediatrician convinced the parents for surgery. As part of pre-op w/u CBC, CMP, UA and PT/PTT were performed. All labs were normal except for **PTT of 40”** (normal 23-33”).

- The pediatrician ordered further w/u for long PTT as per medical school and residency learning.
He is referred to you for further management and advise. What would you do?

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Ref range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTT Mixing study</td>
<td>32</td>
<td>23-33 sec</td>
</tr>
<tr>
<td>FVIII</td>
<td>25</td>
<td>50-150%</td>
</tr>
<tr>
<td>FIX</td>
<td>90</td>
<td>80-120%</td>
</tr>
<tr>
<td>FXI</td>
<td>100</td>
<td>75-100%</td>
</tr>
<tr>
<td>FXII</td>
<td>110</td>
<td>75-100%</td>
</tr>
</tbody>
</table>
Your poll will show here

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Case -1

- No personal and family history of bleeding
- The Doctor’s office is in a suburb
- The sample was drawn in the morning by a nurse, kept at RT before shipping out to a reference lab in late afternoon.
- The patient referred to our center for further evaluation
- Repeat PT and PTT normal – FVIII was 95%
Case -2

- 45 Year old AA male with a diagnosis of SCC of tongue was scheduled for lymph node dissection. The surgeon had ordered PT/PTT. The PTT was 40”
- A hematology consult was placed on Monday.
- PTT mixing study was ordered on Tuesday.
- The mixing study showed PTT of 33” (ref range 23-33.5) on Wednesday.
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Case -2

- FVIII = 45%, FIX = 78%, FXI = 85% and FXII = 100%
- FVIII assay showed an inhibitor pattern
  - With each dilution of PP - FVIII increases by >20%
- Further dilutions of patient plasma showed FVIII = 122%
- The patient had a lupus anticoagulant confirmed by DRVVT
Case 3

- 74 AAM (a JW) presented to the ED with hematuria on Wednesday (PTT = 65”, PT 12”)
- His past Hx included DVT 2 years back.
- PTT at that time was 45” which on mixing study partially corrected to 38”.
- LA was confirmed by DRVVT.
- He was treated with VKA for 1 year.
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He was sent home on Abx for UTI
Friday afternoon presents with macroglossia.
He needed urgent tracheostomy
A hematology consult was ordered along with 6 FFPs for long PTT and some oozing from IV site
What would you do?
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Case -3

- His FVIII was <1% and FVIII inhibitor titer of >250 BU
- Treatment options included:
  - FEIBA (plasma derived)
  - rVIIa
- Given rFVIIa 90 ug/kg 2-4 hours being a JW
- 3 days later space out to 6 hours.
- Bled from tracheostomy wound to Hb 2 g/dl – died
PTT

- Affected by sample collection, hematocrit, processing, transportation, storage
  - Heat labile factors (FV and FVIII)
  - Platelets – can neutralize LA

- Types of PTT reagents used in the lab:
  - PL and contact activator (ellagic acid, kaolin, silica)
  - PTT-FS: factor sensitive (PL +++)
  - PTT-LA: lupus sensitive (PL +)
  - PTT-FSL: factor and lupus sensitive (PL ++)
To Mix or Not to Mix?

- In the past LA was identified by PTT mixing study to differentiate from factor deficiency because there were no diagnostic tests for LA.
- Since mid 1990s diagnostic tests available for LA.
- Therefore, routine mixing study are not clinically helpful and may even harm the patient.
Fallacies of Mixing Study

- Not standardized
- No controls run
- No definition of normal pooled plasma
- No emphasis on platelet free plasma
- Considered to be a routine test and hence performed by any TDH
- No expert supervision or interpretation
Any Indication of Mixing Study?

- Very selected situations
- Only under expert supervision
- Always 2 step PTT mixing
  - 0 hour and 2 hours at 37°C
- Suspected exposure to bovine thrombin
32 year old man with PVD, DMT2, HIV, and HBV presents to ED with increased LLE pain. 
Patient’s LLE pain is associated with coolness and foot swelling. 
CTA reveals occlusion from distal L-superficial femoral artery to the trifurcation of vessels and of R-peroneal artery in distal calf 
Thrombectomy of SF, popliteal and peroneal
<table>
<thead>
<tr>
<th>Post Op Day</th>
<th>Heparin dose IU/hour</th>
<th>PTT Therapeutic range 50-80</th>
<th>Hb g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 and 3</td>
<td>1500</td>
<td>45</td>
<td>10.5</td>
</tr>
<tr>
<td>4</td>
<td>1700-2300</td>
<td>50</td>
<td>9.0</td>
</tr>
<tr>
<td>5</td>
<td>2200-2400</td>
<td>52</td>
<td>7.4</td>
</tr>
<tr>
<td>6</td>
<td>2400-2800</td>
<td>56</td>
<td>6.2</td>
</tr>
<tr>
<td>7</td>
<td>2400-2800</td>
<td>58</td>
<td>9.1 (2 PRBC)</td>
</tr>
</tbody>
</table>

Baseline PTT 21.0 sec (23-33)
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Unpredictable Dose Response of UFH

- Heparin
- ATIII
- Other Plasma Proteins

- Endothelial cell
- Macrophage
- Fibrinogen
- VWF
- Platelets
Case - 4

- UFH anti-Xa activity on day 6 = 1.12 (ref. range = 0.3-0.7) corresponding PTT = 56"'
- AT activity = 62% (ref. range = 84-124%)
- FVIII activity = 319% (ref. range = 50-150%)
- Until day 7, drain output was not recorded
- On POD 7 in the AM the new nurse noted a 10x10 cm hematoma directly superior to the fasciotomy site
True Heparin Resistance

- Increased heparin binding proteins
  - Acute phase reactants
  - Need higher doses of heparin
- Increased FVIII
  - May not need higher dose
  - Monitor with anti-Xa assay
- AT deficiency
  - Perform stat AT levels
  - Infusion of AT
Case -5

- 35 yr HF presents with a spontaneous R- LE DVT
- Started on heparin protocol
- PTTs 70-90” on day 2 (Therapeutic range 50-80”)
- Develops SOB and chest pain on day 2 – PE confirmed
- Admission PT 13.3 (9-12”), INR 1.4 (0.9-1.3) and PTT 45”
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Monitoring anticoagulation in LA

- Baseline prolonged PTT
  - PTT cannot be used for monitoring
  - Heparin assay (anti-Xa assay = 0.3-0.7 U/ml)

- Baseline prolonged PT/INR
  - INR cannot be used to monitor
  - Chromogenic FX (15-30% Therapeutic range)
Case – 5

- The house staff ordered thrombophilia work – up
- LA was confirmed positive and her ACA and Beta 2 GPI IgG were >100 confirming the APS
- Her PS activity was 35% (AT and PC were normal)
- Does she have a concomitant PS deficiency?
- PS: Bound (60%) to C4b and free (40%)
Protein S Activity Assay

PS Deficient Plasma + PP (60% bound & 40% free)

- X
- RVV
- Ca++
- Xa
- PL
- Xa + Va
- Va i
- Ia
- C.T.
- APC
- PS - Cofactor

FVL ➔ False ➔ PS
LA ➔ False ➔ PS
↑ C4b ➔ False ➔ PS
↑ C.T. ➔ PS
Antithrombin Assay

AT (PP) → Heparin Reagent → Thrombin Excess → Free Thrombin

DTI – give false high AT levels

Substrate → Substrate
PC (PP + Protein C deficient Plasma) → Snake Venom → APC

↓ Vₐ

↓ VIIIa

↓ Inactivated D.T.I.

↓ IIₐ

↓ L.A.

↓ False ↑ PC ← ↑ PTT

↓ PTT α ↑ PC

↑ PTT

↑ PC

↓ PC

↑ IIₐ

↓ FVL

↓ Persistence of VIIIa

↓ Persistence of Vₐ int.

↑ IIₐ

↓ PTT

↓ False ↓ PC
Activated Protein C Resistance Assay

1 PP:4 FV Deficient Plasma

\[
\text{APC - Ratio} = \frac{\text{APC + CaCl}_2}{\text{CaCl}_2} = \frac{90}{40} = 2.25
\]

\[
\text{APC - Ratio} = \frac{\text{APC + CaCl}_2}{\text{CaCl}_2} = \frac{70}{40} = 1.75
\]

\[
\text{APC - Ratio} = \frac{50}{40} = 1.25
\]

\[
\text{APC - Ratio} = \frac{85}{60} = 1.5
\]

L.A.
Case – 6

- TMR consulted to manage bleeding in a 66 yrs old cirrhotic patient
- Overnight developed melena
- Hb dropped from 7.0 to 5.9
- Past Medical History
  - COPD
  - Non-alcoholic steatohepatitis (NASH) Cirrhosis
    - MELD on admission: 18
    - Child-pugh class on admission: Class C
    - INR on admission 1.6 and now 1.8
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### The Labs

<table>
<thead>
<tr>
<th></th>
<th>9</th>
<th>8</th>
<th>7</th>
<th>6</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROTIME WITH INR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protime</td>
<td>17.1</td>
<td>18.4</td>
<td>21.5</td>
<td>21.1</td>
<td>21.1</td>
</tr>
<tr>
<td>INR</td>
<td>1.6*</td>
<td>1.8*</td>
<td>2.1*</td>
<td>2.1*</td>
<td>2.1*</td>
</tr>
<tr>
<td>PTT</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>PTT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>45.0*</td>
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<tr>
<td>FIBRINOGEN</td>
<td></td>
<td></td>
<td>195*</td>
<td></td>
<td>110*</td>
</tr>
<tr>
<td>PLATELETS</td>
<td>98</td>
<td></td>
<td>90</td>
<td></td>
<td>93</td>
</tr>
</tbody>
</table>

**ROTEM**
- At the time of ROTEM
  - PT: 18.4 seconds
  - INR: 1.8
  - Partial Thromboplastin Time: 45.0 seconds
  - Fibrinogen: 195 mg/dl
  - Plt 90K
Evaluation of Hemostasis in a Surgically Bleeding Patient

- Current tests have poor TAT and correlation with surgical bleeding
- In a profusely bleeding patient
  - Surgical vs coagulopathic bleeding
- Trauma – multifactorial – hypothermia, acidosis and coagulopathy (consumption, dilutional and ? trauma induced coagulopathy)
- Most non-trauma surgical bleeds in controlled environment (no hypothermia or acidosis) – mostly dilutional coagulopathy
- Obstetrical hemorrhage – mostly surgical since body shifts to hypercoagulable state antepartum – could be consumptive or dilutional
Viscoelastic (?) Point of Care Testing

- Used in trauma and surgical settings to manage bleeding and coagulopathy
  - Thromboelastography (TEG®, Haemonetics, Braintree, MA)
  - Rotational thromboelastometry (ROTEM®, TEM International GmbH, Munich, Germany)
- Allows for real time in-vitro analysis of clot formation, clot strength, and fibrinolysis on whole blood samples.
## Comparison Between Coag tests and VEM

<table>
<thead>
<tr>
<th>Routine Coag</th>
<th>TEG/ROTEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widely available</td>
<td>Not really</td>
</tr>
<tr>
<td>Familiarity</td>
<td>Very unfamiliar</td>
</tr>
<tr>
<td>Interpretation easy</td>
<td>Algorithm dependent and confusing</td>
</tr>
<tr>
<td>Cheap</td>
<td>Expensive</td>
</tr>
<tr>
<td>TAT – 25- 45 min</td>
<td>Rapid 15-20 min</td>
</tr>
<tr>
<td>Platelet free plasma</td>
<td>Whole blood – rheological effect and RBC and plt included</td>
</tr>
</tbody>
</table>
Whole blood:
- Un-anticoagulated
- Citrated

Reagents for:
- Intrinsic pathway
- Heparinase
- Extrinsic pathway (RapidTEG)
- Functional Fibrinogen
- Platelet Mapping
Whole blood:
  - Citrated

Reagents for:
  - INTEM
  - HEPTEM
  - EXTEM
  - FIBTEM
  - APTEM
Viscoelastic Tracing

- **Time**
- **MA/MCF (mm)**
- **angle/α (°)**
- **K/CFT (sec)**
- **R/CT (sec)**
- **LY30/LI30 (%)**
ROTEM® Assays Available

- INTEM—Intrinsic (Surface contact) activation (via Ellagic Acid)
- HEPTEM—adding Heparinase removes heparin from sample (up to 10U/ml)
- EXTEM—Extrinsic activation (via Tissue Factor)
- FIBTEM—adds Cytochalasin D to inhibit platelet contribution
- APTEM—adds aprotinin to inhibit fibrinolysis
ROTEM® Tracing Examples

**EXTEM**
- CT: 67s
- CFT: 87s
- α: 73°
- CFR: 54mm
- MCF: 57mm
- ML: -% 

**INTEM**
- CT: 200s
- CFT: 67s
- α: 77°
- CFR: 54mm
- MCF: 61mm
- ML: -%

**FIBTEM**
- CT: 66s
- CFT: -s
- α: 57°
- CFR: 9mm
- MCF: 10mm
- ML: -%

**APTEM**
- CT: 74s
- CFT: 89s
- α: 72°
- CFR: 53mm
- MCF: 61mm
- ML: -%
Case -6

**EXTEM**

- CT: 64 s [43 - 82]
- CFT: 98 s [48 - 127]
- α: 75 ° [65 - 80]
- A10: 48 mm
- A20: 54 mm [50 - 70]
- MCF: 56 mm [52 - 70]
- ML: * 0 %

**INTEM**

- CT: 143 s [122 - 208]
- CFT: 86 s [45 - 110]
- α: 76 ° [70 - 81]
- A10: 48 mm
- A20: 55 mm [51 - 72]
- MCF: 57 mm [51 - 72]
- ML: * 0 %

**FIBTEM**

- CT: 67 s
- CFT: 72 °
- A10: 15 mm
- A20: 16 mm [7 - 24]
- MCF: 17 mm [7 - 24]
- ML: * 0 %
How to use in clinical settings?
Rapid Interpretation of Results

Assessment using 3 Assay parameters: CT, A10, and ML:

1. $\text{CT}_{\text{IN,EX}}$ = Coagulation factors (heparin on INTEM). severe hypofibrinogenemia or thrombocytopenia.

2. $\text{A10}_{\text{IN,EX}}$ = Clot firmness (platelets, fibrinogen and FXIIIa).
   
   $\text{A10}_{\text{FIB}}$ = Clot firmness (fibrinogen) if normal = platelet problem.

3. $\text{ML}_{\text{IN,EX, FIB}}$ = Clot lysis during the test (Hyperfibrinolysis)
**ROTEM® Guided Bleeding Algorithm**

**INTEM**
- **CT<sub>IN</sub>** > 208
- **CT<sub>HEP</sub>** < 208

**HEPTEM**
- **CT<sub>HEP</sub>** = **CT<sub>IN</sub>

**HEPARIN EFFECT**
- Intrinsic Factor Deficiency

**FIBTEM**
- **A10<sub>IN</sub>** < 41
- **A10 < 9mm**

**Extrinsic Factor Deficiency**

**EXTEM**
- **A10<sub>EX</sub>** < 41
- **CT<sub>EX</sub>** > 82

**Extrinsic Factor Deficiency**

**Consider Surgical Bleeding Source**

**All Normal**

**All Normal**
Cardiac Surgery

INTEM

CT↑>208

HEPTEM

Corrected

Protamine

Not Corrected

Plasma

MCF↓A10<40

FIBTEM

Low < 7

CRYO

>9

Platelets
Trauma/OB Hemorrhage

EXTEM
- CT↑>82
  - Plasma
- ML > 15%
  - APTEM
    - Corrected
      - TXA/AMICAR
- MCF↓A10<40
  - Low <7
    - CRYO
  - N > 9
    - Platelets

FIBTEM
- Low <7
- N > 9
  - Platelets
FFP is not Amrut!

Amrut = Indian mythology “Nectar of Goddess = giving them immortality!”