Fallacies of Coagulation Testing

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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: $>23'' - 35''$
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.
- Pre-op w/u CBC, CMP, UA and PT 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.
<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>

He is referred to you for further management and advise. What would you do?
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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 early in the morning by a nurse, kept at room temperature 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 Squamous Cell Ca of tongue was scheduled for lymph node dissection.
- The surgeon had ordered PT/PTT. The PTT was 38”
- 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 patient plasma - 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 PMH 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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Case 3

- He was sent home on antibiotics for UTI
- Friday afternoon presents with macroglossia.
- He needed urgent tracheostomy
- A hematology consult was placed along with 6 units of 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 (recombinant)
- 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 ++)
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
Case - 4

- 32 year old man with PVD, DMT2, HIV, and HBV presents to ED with increased LLE pain.
- 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

Endothelial cell

= Heparin
= ATIII
= Other Plasma Proteins

= Fibrinogen
= VWF
= Platelets

Macrophage
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
Heparin Resistance

- Increased heparin binding proteins and cells/activity
  - 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-6

- 45 WF with a history of stroke
- Neurologist had ordered thrombophilia work up in out-patient setting
- AT = 155% (80-120%)
- PC = 145% (80-120%)
- PS = 110% (80-120%)
- DRVVT – S = 95”, DRVVT- C = 72”, DRVVT - R = 1.31 (cut off <1.25) = LA positive
- SCT-S = 60”, SCT – C = 55”, SCT-R = 1.09
Case-6

- Neurologist was concerned about weakly positive LA that had a comment stating that anticoagulant therapy may affect LA result.
- He emailed me about this patient being on rivaroxaban and was wondering if that would affect LA test result
## Effects of DOACs on special coagulation tests

<table>
<thead>
<tr>
<th>Assays</th>
<th>IIa inhibitor</th>
<th>Xa-inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixing study (PT or PTT)</td>
<td>May show incomplete correction</td>
<td>May show incomplete correction</td>
</tr>
<tr>
<td>PTT based factor assays</td>
<td>Falsely ↓</td>
<td>May be falsely ↓</td>
</tr>
<tr>
<td>PT based factor assays</td>
<td>May Falsely be low ↓</td>
<td>May be falsely ↓</td>
</tr>
<tr>
<td>Chromogenic FVIII</td>
<td>No effect</td>
<td>Falsely ↓</td>
</tr>
<tr>
<td>AT activity</td>
<td>False ↑ with IIa substrate</td>
<td>False ↑ with Xa substrate</td>
</tr>
<tr>
<td>PC activity – chromogenic</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>PC activity – clot based</td>
<td>False ↑</td>
<td>False ↑</td>
</tr>
<tr>
<td>PS chromogenic</td>
<td>No effect</td>
<td>No effect</td>
</tr>
<tr>
<td>PS - clot based</td>
<td>False elevation ↑</td>
<td>False ↑</td>
</tr>
<tr>
<td>LA</td>
<td>May be false positive</td>
<td>May be false positive</td>
</tr>
<tr>
<td>APCR</td>
<td>False increased ratio</td>
<td>False increased ratio</td>
</tr>
</tbody>
</table>

"false↑" indicates a false increase.
"false↓" indicates a false decrease.
"false↑ with IIa substrate" indicates a false increase with IIa substrate.
"false↑ with Xa substrate" indicates a false increase with Xa substrate.
"may be false positive" indicates may be false positive.
"may show incomplete correction" indicates may show incomplete correction.
"may be falsely ↓" indicates may be falsely decrease.
Case-7

- 54M with Alcoholic Cirrhosis (MELD 22, Childs-Pugh C on admission) presents with altered mental status.
- ? Hepatic encephalopathy (Ammonia 199)
- Some improvement after lactulose but still altered.
- LP by Neuroradiology – want INR <1.5

<table>
<thead>
<tr>
<th>PROTIME W/ INR</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protime</td>
<td>19.8</td>
<td>18.6</td>
<td>18.4</td>
</tr>
<tr>
<td>INR</td>
<td>2.0 *</td>
<td>1.8 *</td>
<td>1.8 *</td>
</tr>
<tr>
<td>PTT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTT</td>
<td>58.2 *</td>
<td>c</td>
<td>61.5 *</td>
</tr>
</tbody>
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No PCC or FFP given because MR Venography showed

In addition no flow void is identified in the superior left ophthalmic vein, suggesting thrombosis. In addition, there is abnormal signal of the left cavernous sinus with no significant enhancement of the left cavernous sinus identified on postcontrast images; this represents a significant change compared to the prior examination performed May 12, 2016. Overall, the findings are consistent with cavernous sinus thrombosis.
Viscoelastic (?) Point of Care (Display) Testing

- To differentiate between surgical vs coagulopathic bleeding
  - Thromboelastography (TEG®, Haemonetics, Braintree, MA)
  - Rotational thromboelastometry (ROTEM®, TEM International GmbH, Munich, Germany)
- Real time in-vitro analysis of clot formation, clot strength, and fibrinolysis on whole blood samples.
Viscoelastic Tracing

- MA/MCF (mm)
- LY30/LI30 (%)
- R/CT (sec)
- K/CFT (sec)

Time
FFP is not Amrut!

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