Changing Coagulation Reagent Lots

BCSLS Congress 2011
Castlegar
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Objective

• Revisit the coagulation model
• Describe the composition of PT and aPTT reagents
• Describe the differences and limitations of different PT and aPTT reagents.
• Outline the procedures involved in evaluating new lot # of PT and aPTT reagents
Coagulation Models

• In-vitro model:
  – Traditional “Coagulation Cascade”.
  – Use in “in-vitro” understanding of coagulation
  – Divides into “intrinsic” and “extrinsic” pathways which involves plasma coagulation factors
In-vitro Model of Coagulation

**INTRINSIC**
(surface contact)

- FXII → FXIIa
- KAL
  - FXI → FXIa
  - F IX → F IXa
  - F VIIIa + PF-3
    - F X → F Xa
      - F Va + PF-3
        - Prothrombin (F II)
          - Thrombin (F IIa)
            - Fibrinogen (F I)
              - Fibrin

**EXTRINSIC**
(tissue damage)

- F VII
  - Tissue factor
  - F VIIa
    - F Xa
      - Thrombin (F IIa)
Cascade Model of Coagulation
Coagulation Models

• In-vivo model:
  – Involves interactions between cellular and plasma components
  – Cellular components include: endothelial cells, fibroblasts, muscle cells, platelets, RBC, lymphocytes and monocytes.
  – Two phases of coagulation:
    • Initiation
    • Propagation
  – Acknowledge the important roles of thrombin and platelets
    – http://www.youtube.com/watch?v=xNZEERMSeyM
Two Phases of Coagulation

- Initiation phase:
  - Act as a “spark” for thrombin formation:

\[ \text{TF/FVIIa} \rightarrow \text{FX} \rightarrow \text{FXa} \rightarrow \text{FIX} \rightarrow \text{FIXa} \rightarrow \text{Thrombin} \]
Propagation Phase

Thrombin

FVIII → FVIIIa

FV → FVa

Ca+

FVIIIa → P FIXa

FX → FXa

Tenase

FV a

Prothrombinase

Prothrombin

Fibrinogen

Fibrin Clot

aPRC → Pr C

aplts → Plts

FXIIIa → FXIII

FXIa → FXI

FVa → FV

FVIIIa → FVIII

FIXa → FIX

Plts

Pr C

FXIII

FXI

FV
Oral Anticoagulant Effect on Coagulation Factors

• Role of Vitamin K
• Oral anticoagulant = Vitamin K antagonist
• Coagulation Factors affected by oral anticoagulant: F II, VII, IX, X
Prothrombin Time Testing

Elements of haemostasis tested by the PT

FIBRINOLYSIS

Black lines indicate inhibitory effect
Prothrombin Time (PT/INR)

- Monitor oral anticoagulant therapy
  - Factors sensitive to OACT: II, VII, IX and X
- Evaluate liver function
- Evaluate hemostastic status prior to surgery, in patient with active bleeding, and patients with bleeding history.
Prothrombin Time

• Most sensitive to Factor VII deficiency
• Sensitive to severe fibrinogen (<1.0 g/L) and Prothrombin (F II) deficiencies.
• Moderate sensitive to F V and X deficiencies
• Insensitive to F VIII and IX deficiencies
Responsiveness of PT reagent
Other conditions that may affect the Prothrombin Time

• Pre-analytic issues:
  – Improper volume
  – 3.8% vs 3.2% sodium citrate
  – Samples with Hct >55%
  – Difficult draw / clotted sample
  – Hemolysed sample

• Direct thrombin inhibitor, unfractionated heparin, LMWH,
• Lupus anticoagulants
• Type of thromboplastin in the PT reagent
Components of Prothrombin Time Reagent

- Thromboplastin Reagent:
  - a phospholipid / tissue factor (human or animal source, recombinant)
  - Calcium ions
  - Stabilizers
  - Buffer
  - Heparin neutralizing agent, e.g. polybrene
Source of Thromoplastin in PT Reagent

– Human source: placenta
– Animal origin: Rabbit brain/lung, ox brain
– Recombinant material

– Each of these sources has limitations that make them problematic in Prothrombin Time determination:
  • Difference in responsiveness to reduction in coag factors
PT Reagent Sources

• Animal brain thromboplastin
  – Lot to lot variability
  – Quality of the phospholipid depends on the extraction process.
  – May have seasonal short supply
  – Political correctness – animal rights

• Human tissue factor
  – Maybe a source of HIV or other viral diseases
  – Short supply

• Recombinant Tissue Factor
Recombinant Tissue Factor

- Recombinant human tissue factor
- Synthetic phospholipids (phosphatidyl choline and phosphatidyl serine)
- Fixed length of fatty acid side chains
- Less lot to lot variability and more standardized sensitivity.
PT Reagents

- 1982: Poller and Taberner:
  - Variable correlation of bleeding complication and dosage of oral anticoagulant
  - Conclusion: Variability was due to the different sensitivities of the thromboplastin reagents used to perform PT.
PT Reagents

1984:

• Proposed standardized reporting of Prothrombin Time, taking in consideration of sensitivity of the thromboplastin reagent.

\[ INR = \left( \frac{PatientPT}{NMPT} \right)^{ISI} \]
Thromboplastin Calibration

- Sensitivities of commercial thromboplastin reagents are calibrated against the International Reference Preparation (IRP) from WHO.
- First IRP has ISI of 1.0
- Other thromboplastins are calibrated against this IRP.
- Reagent with higher ISI is less sensitive.
- We are now on the 3rd generation of IRP
- Calibration of the thromboplastin must be against a reference thromboplastin of the same species, e.g. human against human, rabbit against rabbit etc
WHO Reference Plasma (IRP)

Fig. 18.2 Hierarchy of International Reference Preparation (IRP) for thromboplastin. IRPs coded as small font have already been dismissed.

Kitchen S, Olson J, Preston FE: Quality in Lab Hemostasis and Thrombosis
ISI Calibration

- Protocol for ISI assignments of PT reagents:
- Use plasma from 20 normal F/M, 60 OAC patients stabilized for 6 weeks
- Test with IRP using manual tilt-tube technique
- Repeat the test with testing reagent
- Perform Orthogonal regression analysis
- ISI (testing reagent) = slope x ISI of IRP
Local ISI Calibration of PT Reagent

Test reagent’s ISI = Slope x ISI of the reference plasma
Direct INR Determination

- Obtain a set of certified calibration plasma that have INR determined by IRP.
- Retest the calibration plasma with local reagent and instrument. Determine the PT (sec).
- Plot the local PT (sec) against the certified INR.
- Patient’s INR values is read on this calibration curve.

Advantage of direct INR measurement:
  - Local ISI and NMPT can be calculated

Disadvantage:
  - Lyopholized plasma
  - Assuming linear relationship at high INR
  - Not all coag factors are calibrated, e.g. FV
PT Reagent Evaluation

- Determine local ISI
- Determine Geometric Mean of 20 normal plasma
- Compare patients’ INR using current reagent and new reagent.
- Precision: Run QC at 2 levels over minimum of 10 days.
  - Coefficient of Variance <5 %
- Validate manufacturer’s claim for heparin insensitivity
  - In vitro heparin curve to verify the manufacturer’s claim (up to 1.0 IU/mL)
- Test Factor II, VII and V Sensitivity, if required.
- Determine sensitivity to lupus anticoagulant

Insert spreadsheet here.
Additional Notes on the sensitivity of thromboplastin reagent

- rTF-based commercial thromboplastins (RecombiPlasTin and Innovin) are more sensitive to Factor VII than the tissue-derived reagents (Thromborel-S) and Neoplastine CI Plus) especially at low Factor VII (<10%)

- Different reagent may have different responsiveness to individual vitamin K dependent coagulation factors.

- Reagents that are more responsive to FVII deficiency will show greater drop in INR during the initial oral anticoagulant therapy.

- Thromboplastins of similar ISI do not necessarily respond in a similar fashion to deficiencies in these clotting factors.
Additional Notes on the sensitivity of different thromboplastin reagent

• Factor V and Fibrinogen are not vitamin K dependent but are tested in the PT system. These factors are NOT calibrated.

• Patients with liver failure may not have harmonized INR results using different thromboplastin reagents. This may cause unintentional “queue-jumping” in liver transplant candidates.

• Patients with liver disease generally have greater deficiencies in Factor V and VII than those on oral anticoagulant even they have similar INR results.

• INRs outside the calibration plasma range are not checked. E.g. INR >5.0 need to be interpreted carefully.
Other Limitation of INR Testing

- Under filled or overfilled samples
- Effect of high hematocrit sample (Hct >0.55)
- Lipemic, icteric, hemolysed samples
- Heparin
- Lupus anticoagulant
- Proper storage of reagents
Utility of aPTT Test

• Screening test:
  – Reagent should be sensitive to a reduction in coagulation factors that are commonly associated with bleeding: factor VIII and IX.

• Monitor anticoagulation therapy:
  – Sensitive to unfractionated heparin

• Detection of inhibitors:
  – Lupus anticoagulant is most common.
  – Inhibitors of VIII and IX
aPTT Reagents

- Partial thromboplastin: Contains phospholipids but no tissue factor.
- Phospholipids is a substitute for the membrane of activated platelets
- There are different phospholipids in different reagents
aPTT Reagents

- Has different combinations of phospholipids, activators at different concentrations
- Has instrument dependent clotting time
- Not possible to calibrate
- Has instrument/reagent specific heparin therapeutic ranges and reference intervals
Activators in aPTT Reagents

• Functions
  – Provide a large surface area for reactions to take place
  – Optimize or accelerate the activation of the intrinsic pathway

• Substances used as activators:
  – Glass, silica, kaolin, celite, ellagic acid
Heparin as an anticoagulant

FVIII → FVIIIa

Ca²⁺ → FVIIIa → P → FIXa

FX → FXa

FV → FVa

Prothrombinase

Heparin/AT

Prothrombin

Fibrinogen → Fibrin Clot

Thrombin

aPRC ← Pr C

aPlts ← Plts

FXIIIa ← FXIII

FXIa ← FXI

FVa ← FV

FVIIIa ← FVIII

FIXa ← FIX

Plts

Pr C
Mechanism of Action for Heparin

Heparin: mode of action

Indirect effect on thrombin via AT. Acts like a catalyst in an enzymatic reaction.
Problems with Unfractionated Heparin

- Heparin is negatively charged and binds to different plasma proteins, including the acute phase proteins, resulting in potential under-dosing and variable response from patients to patients.
- Heparin binds to platelet factor 4 and high molecular weight von Willebrand factor from activated platelet or endothelium.
- Heparin can induce thrombocytopenia
Low Molecular Weight Heparin

- Produced by enzymatic or chemical depolymerization of UFH and shortened it into 4 sub groups according to molecular weight size.
- LMWH preparations that has >18 or more saccharides units inhibits both Xa and IIa.
- Preparations that has <18 saccharide units inhibits only Xa.
- The aPTT results decreases according to the size of the LMWH.
aPTT Reagent Evaluation

• Know the type of patients you serve.
• New reagent lot workup:
  – Patients comparison: both normal and abnormal: patients on heparin, factor deficiencies and Las.
  – Heparin sensitivity
  – Factor sensitivity
  – New anticoagulant sensitivity
  – Reference intervals
  – Precision
  – Lupus anticoagulant sensitivity
aPTT New Lot Evaluation

• Patient Comparison Study:
  – Minimum 40 samples, 20 normal, 20 abnormal: heparin, factor deficiencies, LAs

• Heparin Response Curve:
  – In-vitro spiked method is not acceptable.
  – Ex-vivo heparin response study
  – Cumulative Summary for Drift
aPTT Reagent Evaluation

• Precision:
  – Use commercial QC material
  – 20 values from minimum 2 levels
  – CV: <5%

• Reference Interval:
  – Normal donors not on medication
Heparin Response

• Cumulative Summation Method
  – Alternative to ex-vivo anti-Xa
  – Perform anti-Xa the first time.
  – Then use CumSum method for subsequent lot numbers.
CumSum Method for Heparin Response

• Run aPTT on 20 patients on heparin
• Determine sum, mean and SD of results
• Perform cumulative summation of the differences
• Note any shifts in data
• CumSum difference of >7 seconds requires re-establishment of the heparin response curve, or request for a different lot # of reagent.
CumSum Method

<table>
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<tr>
<th>Lot #1</th>
<th>Mean of Old lot</th>
<th>Mean of New lot</th>
<th>Difference (New-Old)</th>
<th>CumSum</th>
<th>Action</th>
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<tr>
<td>1 (04)</td>
<td>78.6</td>
<td>73.9</td>
<td>-4.7</td>
<td>-4.7</td>
<td>Accept</td>
</tr>
<tr>
<td>2a(05)</td>
<td>48.3</td>
<td>41.7</td>
<td>-6.6</td>
<td>-11.3</td>
<td>Reject</td>
</tr>
<tr>
<td>2b(05)</td>
<td>47.6</td>
<td>53.6</td>
<td>+6.0</td>
<td>+1.3</td>
<td>Accept</td>
</tr>
<tr>
<td>3 (06)</td>
<td>71.9</td>
<td>72.3</td>
<td>+0.4</td>
<td>+1.7</td>
<td>Accept</td>
</tr>
<tr>
<td>4a (07)</td>
<td>62.0</td>
<td>71.2</td>
<td>+9.2</td>
<td>+10.9</td>
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</tr>
<tr>
<td>4b (08)</td>
<td>62.8</td>
<td>60.3</td>
<td>-2.5</td>
<td>-0.8</td>
<td>Accept</td>
</tr>
</tbody>
</table>

Determine Heparin Responsiveness in lot #1
Heparin Response Curve

- Heparin Response Curve
- Anti-Xa data must span over the therapeutic range
- Patients must be on Heparin only
aPTT Reagent Evaluation

• Factor Sensitivity:
• Plot factor sensitivity curve
• Should have factor sensitivity around 30%
• Increased levels of some coagulation factors (e.g. VIII) may compensate for other factor deficiencies, and normalize an otherwise prolonged aPTT

• Lupus anticoagulants
  – No single reagent will detect all lupus anticoagulant because of heterogeneity of the LA antibody.
  – Should use different reagent for screening and another for confirmation.
Summary

- PT and aPTT test are assays that must be sufficiently sensitive and robust to fulfill three requirements:
  - Screen for coagulation factor defects
  - Monitor anticoagulant therapy
  - Detect other inhibitors