Consultative Coagulation

How to Effectively Answer Common Questions About Hemostasis Testing

Session #5020

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Disclosures

Dr. (Adcock) Funk and Dr. Moser:

In the past 12 months, I have not had a significant financial interest or other relationship with the manufacturer(s) of the product(s) or provider(s) of the service(s) that will be discussed in my presentation.
Topic Overview

• Direct Oral Anticoagulants
  • Effects on coagulation assays

• Mixing Tests (Mixing Studies)
  • Use and pitfalls

• Case examples will be provided during both course sections
Direct Oral Anticoagulant Agents

*Brief Overview of Effects on Coagulation Assays*
New Oral Anticoagulant Agents

- **Direct Thrombin Inhibitors**
  - Dabigatran
  - Trade Name: Pradaxa®

- **Direct Xa Inhibitors**
  - Rivaroxaban
    - Trade Name: Xarelto®
  - Apixaban
    - Trade Name: Eliquis®
  - Edoxaban
    - Not FDA approved as of yet

Pradaxa® is a registered trademark of Boehringer Ingelheim Pharma GmbH & Co.
Xarelto® is a registered trademark of Bayer Aktiengesellschaft.
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Coagulation Cascade: *In vitro* model

- **Intrinsic pathway**
  - FXII → FIX → FVIII
  - APTT

- **Extrinsic pathway**
  - FXI → FXa → FII
  - PT

- **In vitro model**
  - DXa → FXa → FIIa → Fibrinogen → Fibrin
  - TCT

- **DTI**
Dabigatran Effect on APTT and PT

Dabigatran – TT Response

Rivaroxaban Effect on APTT and PT

Dabigatran and Rivaroxaban (Apixaban) Effect on Coagulation Assays

• Drugs act as inhibitors in the laboratory:
  • Incomplete correction with 1:1 plasma mix
  • Non specific inhibitor effect in factor assays
  • Can cause a false positive Bethesda assay

• False positive Lupus Anticoagulant Assays
• Falsely elevated (normal) APCR, protein C clot-based activity and protein S activity and possibly antithrombin activity (depending on drug and method)

• Dabigatran: Falsely low FXIII activity
• No effect on: D-dimer, VWF assays, free protein S antigen, chromogenic protein C activity, reptilase time
Effect of Dabigatran on Factor Assays

**Intrinsic Factors**

“APTT-Based”

**Extrinsic Factors**

“PT-Based”

Dabigatran and Rivaroxaban Effect on Coagulation Assays

AT assays either IIa or Xa based
PC and PS assays: clot-based

Case # 1

- 33 y/o woman suffers a post-partum DVT and is treated with rivaroxaban. Her physician orders a thrombophilia work-up; patient has the lab work drawn 2 days after starting tx.

- The following assays were performed:
  - Protein S activity (clot based): 65% (63 – 140%)
  - Protein C activity (chromogenic): 72% (55 – 140%)
  - APCR ratio: 2.2 (2.2 – 4.0)
  - dRVVT confirm is positive (ratio 1.3) with negative Staclot® LA and negative aCL and B2GP1 IgG and IgM

Staclot® is a registered trademark of Diagnostica Stago.
Case # 1

• The patient’s clinician calls you to discuss the results and you respond:
  - PS activity may be **falsely (elevated or decreased)** on rivaroxaban?
  - PC chromogenic activity may be **falsely (elevated or decreased)** on rivaroxaban?
  - APCR ratio may be **falsely (elevated or decreased)** on rivaroxaban?
  - LA assay results may be **falsely (positive or negative)** on rivaroxaban?
Case # 2

• 82 year old man with atrial fibrillation is on dabigatran and presents to the ED with bleeding and an elevated APTT and PT. He is in acute renal failure. A FVIII activity with Bethesda titer is ordered:
  - Factor VIII Activity: 8% (50-150%)
  - Bethesda Titer: 1.2 BU (<0.8 BU)

What do you tell the clinician?
Mixing Tests (Mixing Studies)

Use and Pitfalls
Mixing Tests- Overview

• What are plasma mixing tests (mixing studies)?
  • How are they performed?
  • In what situations are they useful?
  • How are they interpreted?
  • Practical examples
Plasma Mixing Test

- Used in the evaluation of a prolonged APTT (most commonly) and/or PT
  - Screen for determining whether prolongation is due to a factor deficiency or inhibitor
  - Can be performed with other assays
    - e.g. DRVVT, VWF activity, FXIII activity

- Performance and interpretation varies
  - Very few published guidelines or standards

The ART of coagulation testing!
Coagulation Cascade: *In vitro* model

- **Intrinsic pathway**: FXII, FXI, FIX, FVIII, FII
- **Extrinsic pathway**: FVII, FV, FX, FII
- **PT** (Prothrombin Time)
- **APTT** (Activated Partial Thromboplastin Time)
- **Thrombin**
- **Fibrinogen**
- **Fibrin**

**TCT** (Thrombin Clotting Time)
Mixing Tests (APTT or PT)

Normal Plasma Mixing Test

**Patient**

↓ APTT or PT

**Normal Pooled Plasma**

**Results**

1. Corrects
2. Fails to Correct

**Principle**- at least ~50% factor is present in the mixture, which is adequate to correct APTTT or PT in cases of factor deficiency
Mixing Tests (APTT or PT)

**Correction** = *Factor Deficiency*

- If immediate correction observed, rule out time/temperature dependent inhibitor

No/partial correction or prolongation with incubation = *Factor Inhibitor*
Mixing Tests - Technical Variables

• Source of normal plasma
• Ratio of patient plasma to normal plasma
  • 1:1 or 4:1 (for a weak inhibitor)
• Method to determine time/temperature dependence
• Criteria for correction
  • Based on specific value versus formula
Source of Normal Plasma (NP)

- Pooled (n ≥ 20) normal plasmas, fresh or frozen
  - Do not use plasma from previously “normal” APTT or PT sample
  - Lyophilized plasmas generally not recommended
- Platelet poor (< 5 -10X 10⁹/L)
- Well characterized
  - Known factor activities (close to 100% of all factors)
  - ↑ FVIII can cause false negative study
  - Should be screened and LA negative
Incubated Mixing Test

Incubate each at 37°C for 60 to 120 minutes, then mix patient with NP.

Separately incubated patient + NP Mix (Control)

Patient + NP incubated together (Incubated Mix)

Patient

NP

50%
Mixing Test Results

What is Correction?
Definitions of Correction

- No consensus or standard definition
- Correction in relation to reference interval
  - Upper limit of 2 SD or 3 SD
  - Upper limit + 5 seconds
- Correction in relation to normal pooled plasma (NP)
  - NP + 5 seconds
  - NP plus 10%
- Correction in relationship to mean normal clotting time
  - Mean normal clotting time + 2SD or +3SD
- Rosner index of \( \leq 15^* \)
- Percent correction*
- Other laboratory-defined criteria

* Index and % correction cutoff must be established by each laboratory
Definitions of Correction, cont.

- Correction in relation to reference range or normal pool
  - Weak inhibitor with minimal prolongation of APTT or PT may correct into normal range with mixing
  - Factor deficiency with prolonged PT may not correct to normal with mix
    - May result from PIVKA interference
- Calculations (% correction or Rosner index)
  - Cut-offs are reagent and instrument specific
  - Each laboratory must determine own cut-offs
Published Calculations for Mixing Study Correction Determination

- Percent Correction

- Rosner Index
Percent Correction


\[
\text{Percent Correction} = \frac{\text{PP APTT – 1:1 Mix APTT}}{\text{PP APTT – CNP APTT}} \times 100
\]

PP – patient plasma; CNP – citrated normal plasma

APTT*
- >75% correction - Factor deficiency
- < 58% correction – Inhibitor

PT*
- > 75% correction – Factor deficiency
- < 70% correction - Inhibitor

*Percent Correction cutoff should be verified by each laboratory
Rosner Index


\[
\text{Index} = \frac{B - C}{A} \times 100
\]

A = Clotting Time of Patient
B = Clotting Time 1:1 Mixing Test
C = Clotting Time of normal pooled plasma (NP)

High index (15 or greater) suggests inhibitor*
Low index suggests factor deficiency*

* Index cut-off must be established by each laboratory
What about minimal prolongation?

- 3-5 s prolonged clotting time
  - Mixing tests are difficult to interpret
  - Are mixing tests indicated?
  - 4:1 APTT plasma mix may be useful
  - Weak inhibitor may correct into the normal range
Follow Up

Prolonged clotting time (PT and/or APTT)

Mixing test (PT and/or APTT, depending on initially prolonged clotting time)

- Corrects: Factor deficiency more likely
  - Appropriate factor activity assays
- Does Not Correct: Inhibitor more likely
  - Lupus anticoagulant and/or specific factor inhibitor assays

Correlation with all available clinical and laboratory information is essential

Time to Practice!
Case 3

<table>
<thead>
<tr>
<th>Test</th>
<th>Result (s)</th>
<th>Reference Interval</th>
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<tbody>
<tr>
<td>PT</td>
<td>12.0</td>
<td>11.9-14.1</td>
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<tr>
<td>APTT</td>
<td>57.2</td>
<td>23.4-36.4</td>
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<tr>
<td>APTT 1:1 mix</td>
<td>33.3</td>
<td>23.4-36.4</td>
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<td>APTT 1:1 incubated</td>
<td>35.2</td>
<td>NA</td>
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<tr>
<td>mix</td>
<td></td>
<td></td>
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<tr>
<td>APTT 1:1 incubated</td>
<td>35.5</td>
<td>NA</td>
</tr>
<tr>
<td>control</td>
<td></td>
<td></td>
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<tr>
<td>TT</td>
<td>11.0</td>
<td>&lt;20</td>
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What are the abnormalities?
What is your differential diagnosis?
What additional testing is indicated?
## Case 4

<table>
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<tbody>
<tr>
<td>PT</td>
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<td>11.9-14.1</td>
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<tr>
<td>APTT</td>
<td>82.2</td>
<td>23.4-36.4</td>
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<tr>
<td>APTT 1:1 mix</td>
<td>58.0</td>
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<td>APTT 1:1 incubated mix</td>
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<td>APTT 1:1 incubated control</td>
<td>68.4</td>
<td>NA</td>
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<tr>
<td>TT</td>
<td>13.6</td>
<td>&lt;20</td>
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What are the abnormalities?
What is your differential diagnosis?
What additional testing is indicated?
# Case 5

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<td>APTT 1:1 incubated mix</td>
<td>44.3</td>
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<td>APTT 1:1 incubated control</td>
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<tr>
<td>TT</td>
<td>9.0</td>
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What are the abnormalities?
What is your differential diagnosis?
What additional testing is indicated?
## Case 6

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<tr>
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What are the abnormalities?
What is your differential diagnosis?
What additional testing is indicated?
Case 7

<table>
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<tbody>
<tr>
<td>APTT</td>
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<td>23.4-36.4</td>
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<tr>
<td>APTT 1:1 mix</td>
<td>48.2</td>
<td>23.4-36.4</td>
</tr>
<tr>
<td>TT</td>
<td>&gt;150</td>
<td>&lt;20</td>
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<td>APTT (heparin neutralized)</td>
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<td>23.4-36.4</td>
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<tr>
<td>TT (heparin neutralized)</td>
<td>&gt;150</td>
<td>&lt;20</td>
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What are the abnormalities?
What is your differential diagnosis?
What additional testing is indicated?
Conclusion- Mixing Tests

• Mixing tests can be a useful screening tool to distinguish the presence of a factor inhibitor from factor deficiency
  • Must be performed and interpreted with caution
  • Guidelines and standards are needed
References – Direct Oral Anticoagulants

References- Mixing Tests


