Chromogenic Factor VIII and IX Assays:

Impact on Diagnosis and Management of Hemophilia

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Disclosure

• None

Objectives

• Recall the types of bleeding disorders
• State the types of hemophilia
• Review the different types of assays: one stage and chromogenic FVIII and FIX
• Discuss the roles and limitations of factor assays in diagnosis and management of hemophilia
Congenital Bleeding Disorders: Types and Approximate Frequency

- ‘Common’ Plasmatic Bleeding Disorders (deficiency)
  - Von Willebrand Disease (von Willebrand factor): affects 1% of the population.
  - Hemophilia
    - A (factor VIII): 1:10,000 live male births
    - B (factor IX): 1:30,000 live male births
    - C (factor XI): 1:100,000 in USA, up to 8% in Ashkenazi Jews.
- Platelet Disorders: rare

Hemophilia A and B: X-linked Recessive

- Classification of Severity based on factor activity levels:
  - Severe hemophilia: <1% (<0.01 IU/mL)
  - Moderate hemophilia: 1%-5% (0.01-0.05 IU/mL)
  - Mild hemophilia: 6%-40% (0.06-0.4 IU/mL)
Role of Measurement of Factor Activity Levels

• Diagnostic: make an accurate diagnosis of subtype of hemophilia
• Prognostic: classify the severity of the disease (and predict severely of bleeding)
• Therapeutic: measurement of post infusion coagulation factor levels

One stage FVIII assay (OSA)

Contact activator
+Ca
+Phospholipid

Intrinsic
XII
XI
IX
VIII
V
X
II
Fibrinogen
Fibrin clot

Extrinsic
VII
PT

End point (clot) detection: Optical vs mechanical

Patient plasma & FVIII deficient plasma
Chromogenic FVIII assay

Human FX vs Bovine FX

Chromogenic Substrate (S-2765)

Stage 1

Ca + Phospholipid + FIXa
Thrombin (optional)

Chromophore group (pNA) + peptide

Stage 2

Patient VIII

Xa

Chromogenic FIX assay

Ca+Phospholipid FXIa + FVIIa
Thrombin (optional)

Patient IX

Chromogenic Substrate (S-2765)

Stage 1

Fx

Stage 2

Xa

Chromophore group (pNA) + peptide
Comparison 1

• One Stage Assay
  • Ability of reference plasma to shorten APTT in hemophilic plasma
  • Physiologic concentrations clotting factors (except FVIII)
  • Simple, rapid, inexpensive, automatable
  • High inter laboratory variation
  • Widely used in USA

• Chromogenic Assay
  • Ability of FVIII to act as cofactor for FIX to activate FX
  • Highly dilute concentrations of clotting factors
  • Factor VIII-deficient plasma not required
  • Lower inter laboratory variation
  • Widely used in Europe

Standard of Care for Severe Hemophilia

• Prophylactic intravenous infusion of factor concentrates (modified and unmodified)

• Unmodified products with standard half-life infusion frequency:
  • rFVIII: approximately 3 times/week
  • rFIX: approximately 2 times/week
    • Frequency of infusion varies with individualized in vivo half-life

• Aim: maintain trough factor level of >1% (1%–5%)

• Dose and frequency of infusions based on pharmacokinetic analysis

• Post-infusion serial measurement of factor levels
Case History #1: New Modified rFIXFc

- 12-year-old male with severe hemophilia B
- Initiated on rFIXFc by hemophilia treatment center
- Dose calculated based on results of pharmacokinetic testing:

<table>
<thead>
<tr>
<th>OSA</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Pre-infusion FIX (trough)</td>
<td>0.05 IU/mL (5%)</td>
</tr>
<tr>
<td>Post-infusion FIX (peak)</td>
<td>0.8 IU/mL (80%)</td>
</tr>
</tbody>
</table>

Case History #1: New Modified rFIXFc (cont)

- Patient was followed in local hemophilia treatment center
- Advised to recheck FIX kinetics
- No bleeding events since initiation of prophylactic rFIXFc infusions
Case History #1: New Modified rFIXFc (cont)

- Rechecked FIX kinetics (in different laboratory)

<table>
<thead>
<tr>
<th></th>
<th>OSA (original)</th>
<th>OSA (recheck)</th>
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<tbody>
<tr>
<td>Pre-infusion FIX</td>
<td>0.05 IU/mL (5%)</td>
<td>&lt;0.04 IU/mL (&lt;1%)</td>
</tr>
<tr>
<td>(trough)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-infusion FIX</td>
<td>0.8 IU/mL (80%)</td>
<td>0.4 IU/mL (40%)</td>
</tr>
<tr>
<td>(peak)</td>
<td></td>
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Case History #1: New Modified rFIXFc (cont)

- Results showed apparent under-recovery or suboptimal FIX dosing (planned to increase the dose of rFIXFc)
- Communication with coagulation laboratory performing assay:
  - Contact activator used for OSA FIX:C in local laboratory: kaolin (underestimates rFIXFc)
  - Mailed out sample to central laboratory performing OSA FIX:C with different contact activator (silica)
    - Confirmed original pharmacokinetic study results
Case History #2: Pharmacokinetic Study With New Modified rFVIII

- 20-year-old male with severe hemophilia A
- Changed from standard rFVIII to new modified rFVIII
- Pharmacokinetic study (30 units/kg) infusion
  - Target FVIII:C approximately 60%

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<tr>
<td>Pre-infusion FVIII:C</td>
<td>&lt;0.01 IU/mL (&lt;1%)</td>
<td></td>
</tr>
<tr>
<td>1 hour Post-infusion FVIII:C</td>
<td>0.3 IU/mL (30%)</td>
<td>0.6 IU/mL (60%)</td>
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OSA, one-stage clot assay.

Case History #2: Pharmacokinetic Study With New Modified rFVIII (cont)

- Results of CSA FVIII

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CSA, chromogenic activity assay; OSA, one-stage clot assay.
Consequences/Risks of Inaccurate Post Infusion Coagulation Factor Measurements

• Underestimation of true factor level
  • Potential consequence:
    • Overdosing; cost and thrombotic complications

• Overestimation of true factor level
  • Potential consequence:
    • Underdosing; increase risk of bleeding and morbidity

Effect of Inaccurate Measurement of Coagulation Factor Levels on the Diagnosis of Hemophilia
Discrepancy Between FVIII and FIX Activity Assays: Potential for Missed Diagnosis\textsuperscript{2,3,4}

- **Severe hemophilia (<1% factor activity):**
  - OSA and CSA are concordant.

- **Non-severe hemophilia (moderate/mild hemophilia A):**
  - Up to 30% of patients: discordant OSA and CSA results
  - OSA may be higher than CSA (more common)
  - CSA may be higher
  - Observed in Patients with specific factor VIII gene mutations.

- **Non-severe hemophilia B**
  - CSA was higher than OSA FIX activity
  - Reflected a milder phenotype

Emicizumab and Coagulation Factor VIII Assays

One stage assay and chromogenic assay with human substrate: false FVIII result in presence of emicizumab.
Implications for FVIII Inhibitor Bethesda Assay

- Using one stage assays and chromogenic assays with human substrate
  - False negative or lower estimates of Bethesda titers
- Performance of Bethesda assay using chromogenic FVIII assays with bovine substrates is required

Conclusions

- Optimal one stage reagent(s) for monitoring of modified factor concentrates vary with molecular modification of protein
- Chromogenic assays provide accurate results for post infusion monitoring of modified, extended half-life recombinant concentrates
- For diagnosis of hemophilia one stage assay may miss or inaccurate classify patients with congenital hemophilia
- For Bethesda titer assessment in patients on emicizumab important to ensure that chromogenic FVIII assays with bovine reagents are being used in the laboratory
References