Hemophiliacs and Factor Inhibitors

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Objectives

• Understand clinical management of inhibitors in hemophiliacs
• Review currently available factor and factor inhibitor assays
• Understand limitations of current testing and need for further standardization
Case

• 5-month-old male with a history of severe Hemophilia A
Classical Hemophilia / Hemophilia A

- X-linked hereditary disorder caused by defective synthesis of FVIII
- Incidence of 1 in 5000-7000 live male births
- Hemorrhagic disease of mild, moderate, or severe severity

<table>
<thead>
<tr>
<th>Severity</th>
<th>FVIII Level (% of normal)</th>
<th>Clinical Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>6-40</td>
<td>Bleeding with trauma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spontaneous bleeding rare</td>
</tr>
<tr>
<td>Moderate</td>
<td>1-5</td>
<td>Bleeding with trauma</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Occasional spontaneous bleeding</td>
</tr>
<tr>
<td>Severe</td>
<td>&lt;1</td>
<td>Spontaneous bleeding into joints or muscles</td>
</tr>
</tbody>
</table>
Factor VIII
Case

• 5-month-old male with a history of severe Hemophilia A
• No history of clinically significant bleeds
• Has an inguinal hernia and undescended testicle
• Requires surgery - how should this be managed?
Products Licensed to Treat Hemophilia A

• Human plasma-derived FVIII concentrates
• Human plasma-derived concentrates containing FVIII and von Willebrand factor (VWF)
• Recombinant FVIII
Factor Replacement Therapy

- Episodic – for symptoms related to bleeding
- Prophylactic – to prevent bleeding
  - Primary – started to prevent clinical bleeding
  - Secondary – started in response to recurrent bleeding
  - Intermittent – for specific circumstances
Case

• Patient will need factor replacement therapy with surgery
• Mother of patient expresses concerns for inhibitor development
What are inhibitors?

- Neutralizing antibodies
  - Interfere with factor activity
  - Decrease factor survival
- The most significant complication of hemophilia treatment
- Substantial increase in morbidity and cost of care
- Occur in up to one third of severe hemophiliacs
- (Some inhibitors are transient, and some do not interfere with factor function)
Risk Factors for Inhibitor Development In Hemophiliacs

- Genetics
  - Severity of disease
  - Genetic mutation responsible for hemophilia
  - Family history
  - Ethnicity
- Treatment
  - Age of first exposure
  - Number of exposure days
  - Type of product
- Other

- Any patient can develop

- Is our case patient at risk?
Case

- Surgery scheduled for 9 months of age
- Treatment with a plasma-derived product containing FVIII and VWF
- FVIII activity measured to assess dosing
FVIII Assays

• One-stage clotting assay (OSA)
  • Serial dilution of test plasma mixed with FVIII-deficient plasma, phospholipid, and surface activator reagents; calcium ions added to start the reaction, and aPTT recorded

• Chromogenic substrate assay (CSA)
  • Stage 1: Incubation of plasma sample with FIXa, FX in excess, thrombin, calcium ions and phospholipid to generate FXa. FVIII is activated, and FVIIIa acts as cofactor for FIXa to convert FX to Fxa
  • Stage 2: Determine amount of FXa produced. FXa hydrolyzes a chromogenic substrate and color intensity of product is directly proportional to the amount of FXa (and FVIII) in the sample
FVIII Assays

- OSA
  - Widely used in the US
  - Rapid, inexpensive, easy to automate
  - Susceptible to interference from pre-activation of FVIII and lupus inhibitor

- CSA
  - Not as widely used
  - More expensive
  - Not susceptible to pre-activation of FVIII, insensitive to lupus inhibitor
  - Lower inter-laboratory CVs

- Some mild hemophilia variants only picked up by one or the other
- Discrepancies in measurement of recombinant factor products
Case

• Successful surgery
• Receives 6 days total of therapy for surgery and post-operative course
• Needs testing to monitor for inhibitor development
Bethesda Inhibitor Assay

- Comparison of the amount of factor in the test reaction with the control reaction reflects the strength of the inhibitor
- 1 Bethesda unit = amount of inhibitor that results in 50% residual activity
- Residual activity = (Patient mix/control mix) x 100
- Convert residual activity to Bethesda unit
- Adjust for dilution as needed
Calculation of Bethesda Units

- Read from semi-logarithmic plot of Bethesda units (linear) versus residual FVIII activity (logarithmic)
Nijmegen Modified Bethesda Assay

- Control is more similar to patient sample
- Buffering decreases inactivation of FVIII in unbuffered normal plasma
- Recommended by ISTH

**Diagram:**
- Patient Plasma
- Buffered Normal Plasma
- FVIII Deficient Plasma
- Buffered Normal Plasma

- Incubate 2 hrs at 37°C
- Perform FVIII Assay
- Calculate Residual Activity
- Calculate Inhibitor Units
(Some of the) Additional Functional Methods

• Hybrid of Bethesda and Nijmegen
• Nijmegen with 4% bovine serum albumin instead of FVIII deficient plasma
• Heat inactivated
  • No need for “wash out”
  • Heat patient and control plasmas to 56°C for 30 minutes and centrifuge
• Chromogenic
  • Not influenced by non-specific inhibitors
• Low titer inhibitor method
  • Lower limit of detection (0.03 vs 0.6)
  • Patient plasma concentrated, higher ratio to normal pooled plasma, chromogenic
What assay does your lab use?

- Bethesda
- Nijmegen
- Hybrid
- Heat inactivated
- (Chromogenic)
Immunoassay Methods

• Enzyme linked immunosorbent assay (ELISA)
  • Antibodies bind to FVIII immobilized on a surface

• Fluorescence-based immunoassay (FLI)
  • Antibodies bind to FVIII immobilized on fluorescent beads
Immunoassay Methods

- More sensitive
- Not specific for neutralizing antibodies
- May have prognostic value
Case

- Unfortunately, testing 13 days after factor exposure reveals...
- FVIII activity <1%
- FVIII Inhibitor 59 BU
Treatment of Inhibitors

• Titer classified as low or high relative to 5 BU
  • Low responders when the titer is persistently <5 BU
  • High responders when the titer has been ≥5 BU/ml at any time

• Management of Bleeding
  • Titer
    • Titer <5 BU => continue treatment with FVIII concentrates
    • Titer ≥5 BU => treat with FVIII bypassing agents
  • Bypassing agents
    • FEIBA - Factor VIII Inhibitor Bypassing Activity
      • Contains mainly non-activated II, IX, and X, and mainly activated VII
    • FVIIa recombinant
Immune Tolerance Induction (ITI)

• High doses of FVIII product given regularly to create tolerance and decrease titer

• Recommended for:
  • Patients with confirmed titers of ≥5 BU and ≤10 BU
  • Patients with higher titers with ongoing bleeding

• Predictors of success
  • Low historical peak inhibitor titer
  • Titer <10 BU before initiation of ITI
  • Lower titer during ITI
  • Younger age and/or therapy initiated <5 years after inhibitor diagnosis

• Immunomodulation may be used if refractory
  • Rituximab: anti-CD20 monoclonal antibody
Case

• Last FVIII Inhibitor 25 BU
• Plan to initiate ITI when <10 BU or earlier if clinically required
Case

Hematologist calls: “My patient has a very different inhibitor titer at a different lab. Which titer should I use to monitor him? I don’t know when to start immune tolerance induction.”

...good question
Variability in Inhibitor Measurements

• Inter-laboratory CVs often approach (and sometimes exceed) 50% (Favaloro et al.)
• Many sources of variability
  • Method
  • Reagents
  • Technical preparation of patient plasma dilutions
  • Calculation of Bethesda Unit
A Second Case

• 17 year old male
• History of easy bruising and hemarthroses in childhood led to diagnosis of hemophilia B (FIX levels of 1-2%)
• Receiving prophylaxis with recombinant FIX
• Worsening right knee pain and swelling despite increased dosing of recombinant FIX at home
Hemophilia B

- X-linked hereditary disorder caused by defective FIX
- Incidence of 1 in 25,000-30,000 live male births
- Also a hemorrhagic disease of mild, moderate, or severe severity
- Inhibitors in Hemophilia B are rare and develop in around 3% of patients with severe disease
Case

- FIX Activity <1%
- FIX Inhibitor 14 BU
Case

• How should this patient be managed?
Case

• Management of bleeding
  • FIX infusions will not stop
  • Bypassing agent is indicated

• Long term management of inhibitor
  • No clear, evidence-based guidelines due to rarity of disease
  • Possibilities: living with inhibitor, ITI therapy, ITI therapy with immune modulation
Need for Standardization

• Clinical Management
  • The earlier an inhibitor is detected, the better the outcome of therapy
  • Inadequate monitoring leaves patients at risk of bleeding complications
  • Prognosis
  • Treatment decisions

• Research
  • Ongoing assessment of risk of factor products
  • Assessment of other inhibitor risk factors
  • Ongoing development of treatment guidelines
Conclusions

• Factor and factor inhibitor measurements are essential for the care of hemophilia patients
• Standardization has not yet been achieved
• Clinicians and laboratorians need to be aware of the differences and potential limitations of these assays to correctly care for patients

Matthew Jajeh, a 16-year-old in Millbrae, Calif., learned to give himself injections of a clotting drug by practicing on a teddy bear. “Hemophilia really made me grow up a lot faster than other kids,”
References

• Favaloro et al. Laboratory testing for factor inhibitors. Haemophilia 2014.
• Miller et al. Validation of Nijmegen-Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. Journal of Thrombosis and Haemostasis 2012.