Inhibitor Results and Dilemmas in Inhibitor Testing

Amanda B Payne, MPH
Biologist
Division of Blood Disorders
Centers for Disease Control and Prevention

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What is an inhibitor?
What is an inhibitor?

Knesek et al., Thrombosis 2012
What is an inhibitor?

WITH APPROPRIATE TREATMENT CLOT FORMS

Knesek et al., Thrombosis 2012
What is an inhibitor?

Factor VIII Inhibitor\(^1\)

1: Antibody directed against Factor VIII treatment product

Factor IX Inhibitor\(^2\)

2: Antibody directed against Factor IX treatment product
Inhibitors

- **Frequency**
  - Hemophilia A: up to 30%
  - Hemophilia B: <5%

- **At risk**
  - All severities
    - More common in severe hemophilia
  - All ages
    - Peaks with early exposures to factor and late in life

- **Can lead to increased**
  - Product utilization
  - Healthcare costs
  - Morbidity
  - Mortality

1: Wright J, Paisley S 2003
2: Armstrong EP 2014
3: Guh S 2008
4: Walsh CE 2015
Testing for Inhibitors

- **Functional**
  - Measures ability of patient plasma to clot compared to normal plasma
  - Clot-based endpoint
  - Chromogenic endpoint

- **Immunologic**
  - Measures presence of antibodies
  - Enzyme-linked immunosorbent assay (ELISA)
  - Fluorescence immunoassay (FLI)
Testing for Inhibitors

- **Functional Assays**
  - Patient plasma containing FVIII inhibitor
  - Plasma from healthy donor
  - Test reaction
  - Control reaction
  - Less clot formation
  - More clot formation

- **Immunologic Assays**
  - Factor VIII or IX
  - Covalently couple
  - Incubate with plasma samples
  - 1. anti-human antibody
  - 2. reporter
Need for Centralized Inhibitor Testing

- Inter-laboratory variability in results\(^1\)
  - Documented by international proficiency testing programs
  - Between-laboratory CVs near 50%
  - False positives: up to 32% on known specimens

- Methodologic differences among laboratories\(^2\)
  - Among 53-78 NASCOLA laboratories in US and Canada 2010-12
    - 20% using the Nijmegen assay, 10% using the Bethesda assay
    - 70% hybrid assay

- Variability in inhibitor testing practices\(^3\)

- Physician concerns
  - Test validity
  - Labelling of patients
  - Requirement for washout

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Informing Centralized Inhibitor Testing - Needs

- Need testing methods that are...
  - Valid
  - Consistent
  - Avoid wash-out
  - Avoid false-positives
Informing Centralized Inhibitor Testing - Test Methods

Patient plasma

Test reaction

Pooled Normal plasma

Control reaction

Incubate @ 37° for 2 hours

Measure FVIII

Patient mix/control mix X 100 = % residual activity (RA)

Convert RA to BU by formula: 
\[ BU = (2 - \log RA) \cdot (0.301)^{-1} \]

Adjust for dilution, if necessary

BU, Bethesda units.

Informing Centralized Inhibitor Testing - Test Methods

- **Test reaction**
  - Patient plasma
  - Measure FVIII
  - Patient mix/control mix × 100 = % residual activity (RA)
  - Convert RA to BU by formula: BU = (2 - log RA)(0.301)^{-1}
  - Adjust for dilution, if necessary

- **Control reaction**
  - Imidazole buffer

- **Valid**
- **Consistent**
- **Avoid wash-out**
- **Avoid false-positives**

BU, Bethesda units.
Informing Centralized Inhibitor Testing - Test Methods

Test reaction

Patient plasma

Buffered Pooled Normal plasma

Incubate @ 37° for 2 hours

Measure FVIII

Control reaction

Patient mix/control mix X 100 = % residual activity (RA)

Convert RA to NBU by formula: NBU=(2-log RA)(0.301)^{-1}.

Adjust for dilution (hemophilic plasma), if necessary

Informing Centralized Inhibitor Testing - Test Methods

- **Test reaction**
  - Patient plasma
  - Measure FVIII
  - Patient mix/control mix \( \times 100 = \% \) residual activity (RA)
  
  - Convert RA to NBU by formula: 
  
  \[
  NBU = (2 - \log RA)(0.301)^{-1}
  \]
  
  - Adjust for dilution (hemophilic plasma), if necessary

- **Control reaction**
  - Hemophilic plasma

  - Valid
  - Consistent
  - Avoid wash-out
  - Avoid false-positives
Informing Centralized Inhibitor Testing - Test Methods

**Test reaction**

- Patient plasma heated to 56°C for 30 min.
- Buffereed Pooled Normal plasma
- Incubate @ 37°C for 2 hours
- Measure FVIII

**Control reaction**

- Hemophilic plasma

Patient mix/control mix × 100 = % residual activity (RA)

Convert RA to NBU by formula: NBU = (2 - log RA)(0.301)^{-1}. Adjust for dilution (hemophilic plasma), if necessary

Informing Centralized Inhibitor Testing - Test Methods

Test reaction

- ✔ Valid
- ✔ Consistent
- ✔ Avoid wash-out
- ❏ Avoid false-positives

Control reaction

Hemophilic plasma

Patient plasma heated to 56° for 30 min.

Measure FVIII

Patient mix/control mix X 100 = % residual activity (RA)
Convert RA to NBU by formula: 
\[ \text{NBU} = \frac{2 - \log \text{RA}}{0.301} - 1 \]

Adjust for dilution (hemophilic plasma), if necessary

Informing Centralized Inhibitor Testing - Methods

- Positive clotting assay results:
  - **Clinically significant**: factor treatment ineffective
  - **Transient**: usually low titer (<2.0 NBU), factor treatment usually remains effective, titer declines within months without special treatment
  - **False positives**: usually low titer (<2 NBU); factor treatment remains effective; subsequent tests will be negative
    - Heparin
    - Lupus anticoagulants
    - Non-specific inhibitors
Informing Centralized Inhibitor Testing - Test Methods

**Test reaction**
- Patient plasma heated to 56°C for 30 min.
- Incubate @ 37°C for 2 hours
- Measure FVIII or IX by clotting or chromogenic assay
- Patient mix/control mix X 100 = % residual activity (RA)
- Convert RA to NBU by formula: NBU = (2 - log RA)(0.301)^{-1}
- Adjust for dilution (hemophilic plasma), if necessary

**Control reaction**
- Buffered Pooled Normal plasma
- Incubate @ 37°C for 2 hours
- Hemophilic plasma

Informing Centralized Inhibitor Testing - Methods

1. biotinylated anti-human Ig
2. PE-streptavidin

Carboxylated Polystyrene Bead

covalently couple
Factor VIII or IX

Coupled polystyrene bead

incubate beads with plasma samples

1: Boylan B 2015
2: Boylan B 2016
Informing Centralized Inhibitor Testing - Methods

Informing Centralized Inhibitor Testing - Methods

Modified Nijmegen-Bethesda Assay (All)

- **FVIII:** <0.5 NBU
  - **FIX:** <0.3 NBU (Negative)
  - Repeat NBA
  - Chromogenic NBA (FVIII)
- **FVIII:** 0.5 ≤ NBU < 2.0
  - **FIX:** 0.3 ≤ NBU < 1.0 (Low Positive)
  - FLI
- **FVIII:** ≥ 2.0 NBU
  - **FIX:** ≥ 1.0 NBU (Positive)
  - DRVVT
Informing Centralized Inhibitor Testing - Methods

Modified Nijmegen-Bethesda Assay

- Valid
- Consistent
- Avoid wash-out
- Avoid false-positives

FVIII: <0.5 NBU
FIX: <0.3 NBU
(Negative)

FVIII: 0.5≤NBU<2.0
FIX: 0.3≤NBU<1.0
(Low Positive)

FVIII: ≥2.0 NBU
FIX: ≥1.0
(Positive)

Repeat NBA
Chromogenic NBA (FVIII)
FLI
DRVVT
Chromogenic, FLI, and DRVVT results

**CDC Modified Nijmegen Bethesda Assay result**

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Units</th>
<th>Negative Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor VIII inhibitor</td>
<td>1.2</td>
<td>Nijmegen-Bethesda units</td>
<td>&lt; 0.5 NBU</td>
</tr>
</tbody>
</table>

**Reason for Specimen Draw**

Initial Surveillance Inhibitor Titer

**Comments:** This specimen was also positive by chromogenic Bethesda assay at 1.4 CBU and positive for anti-PVIII antibodies by fluorescence immunoassay, performed as described in the references cited below. DRVVT was negative.

If any test results are different than current records indicate, please contact Dr. Connie Miller at (404) 639-2851.

The effects of new longer acting treatment products on inhibitor tests are unknown. False negative results may result. Bypassing agents and ACE910 (Emicizumab) are known to interfere with the CDC Nijmegen Bethesda Assay.

A single positive test is not diagnostic of an inhibitor. False positive tests are known to occur, particularly for factor VIII inhibitors in the range of 0.5-1.9 NBU. Any unexpected positive test should be redrawn for confirmation. Diagnosis of an inhibitor is a clinical judgment to be made by the patient’s physician using additional clinical information, such as recovery and half-life of infused treatment product. An inhibitor would be expected to lead to a reduced recovery and/or shortened half-life.

Specimens were tested by the methods described in Miller et al. J Thromb Haemostasis 2012; 10: 1055-61, Miller et al. J Thromb Haemostasis 2013; 11: 1300-9, Boylan et al. J Thromb Haemostasis 2015; 13: 47-53, and Boylan et al. J Thromb Haemostasis 2016; 14: 1931-40. These are Laboratory Developed Tests, which have not been cleared or approved by the FDA. The performance characteristics have been established by the CDC Hemostasis Laboratory.
Centralized Inhibitor Testing – Factor VIII Results

Modified Nijmegen-Bethesda Assay
8163 tests

<0.5 NBU
95%

0.5-1.9 NBU
2%

≥2.0 NBU
3%

Confirmatory Tests

Negative
0.1%

Ambiguous
0.7%

Positive
1.2%

Re-evaluate next year

Request Repeat Specimen
Centralized Inhibitor Testing – Factor VIII Results

Follow Up on FVIII Specimens

50 Specimens with Negative History Reported

34 Specimens with Negative History Confirmed

- Not Confirmed 7 (21%)
- Confirmed 13 (38%)
- Pending 14 (41%)
Centralized Inhibitor Testing – Factor IX Results

Modified Nijmegen-Bethesda Assay 2015 tests

- <0.5 NBU 97.4% (Re-evaluate next year)
- 0.3-0.9 NBU 1.2%
- ≥1.0 NBU 1.4% (Request Repeat Specimen)
Centralized Inhibitor Testing – Factor IX Results

Follow Up on FIX Specimens

10 Specimens with Negative History Reported

10 Specimens with Negative History Confirmed

Not Confirmed
5 (50%)

Confirmed
3 (30%)

Pending
2 (20%)
If any test results are different than current records indicate, please contact Brandi Dupervil at (404) 498-6879.

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Dilemmas in Centralized Inhibitor Testing – Novel Products

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## FVIII or FIX Deficiency
- Anti TFPI antibody
- AT3 Inhibitor
- Fc fusion
- PEGylation
- Albumin fusion
- Single chain
- Engineered bivalent antibody

## Inhibitor
- Engineered bivalent antibody
- Albumin fusion
- PEGylation
- CTP fusion
Dilemmas in Centralized Inhibitor Testing – Novel Products

FVIII or FIX Deficiency

- Anti TFPI antibody
- AT3 Inhibitor
- Fc fusion
- PEGylation
- Albumin fusion
- Single chain
- Engineered bivalent antibody

Inhibitor

- Engineered bivalent antibody
- Albumin fusion
- PEGylation
- CTP fusion
Dilemmas in Centralized Inhibitor Testing – Novel Products

- ACE910 (Emicizumab)
  - Mimics the action of activated factor VIII
  - Completely unrelated structurally
  - Half-life of 4-5 weeks
  - Not readily heat-inactivated

Dilemmas in Centralized Inhibitor Testing – Novel Products

- **ACE910 (Emicizumab)**
  - Must start with “follow-up” tests

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**Modified Nijmegen-Bethesda Assay**

- **FVIII**: $<0.5 \text{ NBU}$
  - **FIX**: $<0.3 \text{ NBU}$
  - (Negative)

- **FVIII**: $0.5 \leq \text{NBU} < 2.0$
  - **FIX**: $0.3 \leq \text{NBU} < 1.0$
  - (Low Positive)

- **FVIII**: $\geq 2.0 \text{ NBU}$
  - **FIX**: $\geq 1.0 \text{ NBU}$
  - (Positive)

- **Repeat NBA**
- **Chromogenic NBA (FVIII)**
- **FLI**
- **DRVVT**
Maintaining the Accuracy and Validity of Centralized Inhibitor Testing

Factor Deficient Plasma  →  Treatment Product

Heat 56°C for 30 Minutes

Factor Activity

\[ \text{t}_0 \rightarrow \text{t}_10 \rightarrow \text{t}_20 \rightarrow \text{t}_30 \]
Maintaining the Accuracy and Validity of Centralized Inhibitor Testing

Impact of method validation studies:
• Provide information regarding effect of heat treatment on interfering factor product for new products
• IF product-specific differences identified:
  • Evaluate different heat treatment algorithms
    • More time?
    • Higher heat?
  • Use chromogenic or FLI as “first-line” assay
Maintaining the Accuracy and Validity of Centralized Inhibitor Testing

- **Let us know** (preferably before you send the specimen) if a patient has recently infused with a novel treatment product
- Be on the look-out for **updates** to the Community Counts Registry Specimen Form
  - Currently prescribed medications
  - Date product infused last (for each currently prescribed medication)
Centralized Inhibitor Testing - Reminders

- As a minimum, **yearly inhibitor testing** should be part of routine standard of care management for all individuals with hemophilia. Testing should be conducted more frequently as clinically indicated.

- When an **inhibitor is suspected**, a **specific** Bethesda Assay (BA) or Nijmegen-Bethesda Assay (NBA) should be performed immediately in a local laboratory, and **confirmatory testing** of inhibitor titers **less than 2.0 NBU** by chromogenic Bethesda Assay (CBA) or **immunologic test**, such as ELISA, should be conducted. **If any of these tests is not available locally, the specimen can be sent to CDC Division of Blood Disorders, Hemostasis Laboratory**.

- CDC’s Community Counts should be promoted as a program that encourages regular inhibitor testing and NHF’s My Life, Our Future for the collection of genotyping data to assess inhibitor risk. These programs provide valuable contributions to individuals’ understanding of their condition as well as research and public health insight into the hemophilia population as a whole. MASAC encourages all HTCs and all consumers to participate in these programs.
Centralized Inhibitor Testing - Reminders

- **Community Counts Inhibitor Testing**
  - Eligible Diagnoses:
    - Hemophilia A
    - Hemophilia B
    - Type 3 VWD
  - Treated with clotting factor concentrate or any blood products
    - Initial surveillance visit: ever treated or unknown treatment history
    - Subsequent surveillance visit: treated since last surveillance visit
  - Follow-up to positive result
    - Detected at CDC OR locally
  - No washout period from factor use is necessary when collecting specimens for inhibitor testing
  - “Excess” plasma collected for other reasons may be submitted for this project

- **Specialized Inhibitor Testing**
  - Chromogenic and FLI testing to follow-up suspected positive
  - Regardless of Community Counts enrollment
Centralized Inhibitor Testing - Reminders

- **Community Counts Inhibitor Testing**
  - Eligible Diagnoses:
    - Hemophilia A
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    - Detected at CDC OR locally
  - No washout period from factor use is necessary when collecting specimens for inhibitor testing
  - “Excess” plasma collected for other reasons may be submitted for this project

- **Specialized Inhibitor Testing**
  - Chromogenic and FLI testing to follow-up suspected positive
  - Regardless of Community Counts enrollment

regardless of age!

we want to confirm!
Conclusions

- CDC utilizes a variety of inhibitor testing methodologies to provide valid, consistent centralized inhibitor testing that attempts to avoid false positive results
- As novel products come to the market, a change in testing strategy may be necessary (we are currently investigating this)
  - Let us know if a patient is on a novel treatment product
  - Look for updates to the Specimen Form
- Enrollment in the Community Counts Registry is a great way to follow NHF’s MASAC recommendations for inhibitor testing
- Patients with Hemophilia A, Hemophilia B, and Type 3 VWD are eligible for inhibitor testing through enrollment in the Community Counts Registry
  - All ages
  - Received blood products or unknown treatment history
- Specialized testing is available to confirm low positives (regardless of Community Counts enrollment)
Conclusions

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For more information, contact CDC
1-800-CDC-INFO (232-4636)

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.