Factor VIII Inhibitors: Advances in Basic and Translational Science

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CIRCULATING anticoagulants may be defined as "abnormal endogenous components of blood which inhibit the coagulation of normal blood." An anticoagulant directed against antihemophilic factor has been recognized for several decades. This inhibitor and others directed against the various stages of coagulation have been the subject of recent extensive reviews.

Investigation of the mode of action of anticoagulants has lagged behind clinical description because of the lack of simple, reproducible, in vitro assay systems for the factors being measured. Recently Biggs and her associates have applied the thromboplastin generation test to the study of the anticoagulant directed against antihemophilic factor and have described some of the kinetics of the anticoagulant-antihemophilic factor reaction.
Factor VIII Inhibitor Incidence in Severe Hemophilia A Previously Untreated Patients (PUPs)

Inhibitor incidence ~30%

Median FVIII exposure days 10 - 20

Median age 1 - 2 years
Factor VIII Inhibitor Incidence in Previously Treated Patients

2.14 cases per 1,000 person years
(Kempton et al. UDC data)

3.2 cases per 1,000 person years
(Darby et al. UK Registry)
FVIII Inhibitor Incidence in Non-Severe Hemophilia A

Eckhardt et al. Blood 2013
Influences on FVIII Inhibitor Development

**Genetic**
- Family history
- Ethnicity
- FVIII genotype
- HLA genotype
- TNFα genotype
- IL-10 genotype
- CTLA4 genotype

**Environmental**
- Intensity of FVIII therapy
- Continuous FVIII infusion
- Time of therapy initiation
- Co-existent inflammation
- Type of FVIII product
- Product Switching

**Complex and Multifactorial**
Influence of F8 Mutations on FVIII Inhibitor Incidence

Gouw et al. Blood 2010
Enhanced Inhibitor Risk F8 Mutations in Non-Severe Hemophilia A

Inhibitor Risk at 50 EDs

- Trp2229Cys: 41.7%
- Arg2159Cys: 39.4%
- Asp2074Gly: 21.2%
- Arg593Cys: 18.3%
## Factor VIII Inhibitor Predictive Factors (Immunogenotypic Influences)

<table>
<thead>
<tr>
<th>Cytokine/cytokine receptor promoter polymorphisms</th>
<th>OR</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>IL-10</td>
<td>5.4</td>
<td>(2.1 – 13.7)</td>
</tr>
<tr>
<td>TNFα</td>
<td>4.0</td>
<td>(1.4 – 11.5)</td>
</tr>
<tr>
<td>CTLA4</td>
<td>0.3</td>
<td>(0.1 – 0.8)</td>
</tr>
</tbody>
</table>

The Polygenic Nature of Inhibitors in Hemophilia A

Results from the Hemophilia Inhibitor Genetics Study (HIGS) Combined Cohort

- 833 persons with hemophilia
  - Hemophilia Inhibitor Genetics Study (HIGS) (448)
  - Malmo International Brother Study (MIBS) (120)
  - Hemophilia Growth and Development Study (HGDS) (265)

- 13,331 SNPs from 1,801 immune response/modifier genes

Astermark et al. Blood 2013;121(8):1446-54
Hemophilia Inhibitor Genetics Study
combined cohort

Results

• 53 SNPs predictors of inhibitor status
  – Odds ratio in same direction in all 3 study cohorts

• 8 (of the 53) SNPs significant predictors in discordant pairs

Astermark et al. Blood 2013;121(8):1446-54
Complex Human Traits: Results from Genome-Wide Association Studies

**Acquired "Immune" Diseases**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Approx Number of Genetic Loci</th>
<th>Approx Number of Study Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn's Disease</td>
<td>61</td>
<td>6,000 / 15,000</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>74</td>
<td>14,000 / 44,000</td>
</tr>
</tbody>
</table>

Pickrell et. al. Nature Genetics July 2016
Do Recombinant FVIII Products Possess a Higher Immunogenic Risk?
USE OF RECOMBINANT ANTIHEMOPHILIC FACTOR IN THE TREATMENT OF TWO PATIENTS WITH CLASSIC HEMOPHILIA

GILBERT C. WHITE, II, M.D.,
CAMPBELL W. MCMILLAN, M.D.,
HENRY S. KINGDON, M.D., PH.D.,
AND CHARLES B. SHOEKER, PH.D.

COMMERCIAL concentrates of human antihemophilic factor (factor VIII) have been available for nearly 20 years and have resulted in dramatic changes in the treatment of classic hemophilia. Home therapy, surgical intervention, and prolonged treatment of hospitalized patients with hemophilia have all been facilitated by the availability of lyophilized factor VIII preparations.

Although factor VIII concentrates have improved the ease and effectiveness of treatment, transfusion-associated complications have also increased. Patients with hemophilia have a high frequency of seropositivity for human immunodeficiency virus type 1 (HIV-1) and are at risk for the acquisition of the acquired immunodeficiency syndrome (AIDS). Most reports indicate that 60 to 80 percent of patients with hemophilia who were exposed to factor VIII concentrates between 1979 and 1984 are seropositive for HIV by Western blot assay.1-5 As of May 1988, more than 639 patients with hemophilia had AIDS.6 In addition, approximately 80 percent of the patients with hemophilia who require transfusions are seropositive for antibody to hepatitis B virus, and 25 percent have chronic liver dysfunction.7-12 Between 5 and 10 percent of such patients are positive for hepatitis B surface antigen. Both short-incubation and long-incubation forms of non-A, non-B hepatitis have also been reported in patients with hemophilia, as has delta hepatitis,13-17 but the frequency of these disorders is difficult to estimate without serologic markers.

Techniques for heat inactivation of HIV in factor VIII concentrates have been developed18,19 and have reduced the infectivity of the concentrates. Donor screening for HIV is certain to reduce the infectivity of these products further. Nevertheless, sporadic reports of seroconversion and febrile illnesses similar to acute HIV infections continue to be reported in patients
Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review

Iorio et al. Journal of Thrombosis and Haemostasis June 2010 Vol 8
Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review

2,094 hemophilia A PUPs  -  24 Studies

Pooled FVIII Inhibitor Rates

14.3%  pdFVIII

27.4%  rFVIII

(High Responders:  9.3% pd FVIII,  17.4% rFVIII)

Iorio et al.  Journal of Thrombosis and Haemostasis June 2010 Vol 8
Rate of inhibitor development in previously untreated hemophilia A patients treated with plasma-derived or recombinant factor VIII concentrates: a systematic review

Multiway ANOVA Analysis

Study period
Testing frequency
Median follow up

Explained most of the difference

Source of Concentrate - lost statistical significance

Iorio et al. Journal of Thrombosis and Haemostasis June 2010 Vol 8
Factor VIII Products and Inhibitor Development in Severe Hemophilia A

Samantha C. Gouw, M.D., Ph.D., Johanna G. van der Bom, M.D., Ph.D.,
Rolf Ljung, M.D., Ph.D., Carmen Escuriola, M.D., Ana R. Cid, M.D.,
Séguolène Claeysens-Donadel, M.D., Christel van Geet, M.D., Ph.D.,
Gili Kenet, M.D., Anne Mäkipernaa, M.D., Ph.D., Angelo Claudio Molinari, M.D.,
Wolfgang Muntean, M.D., Rainer Kobelt, M.D., George Rivard, M.D.,
Elena Santagostino, M.D., Ph.D., Angela Thomas, M.D., Ph.D.,
and H. Marijke van den Berg, M.D., Ph.D.,
for the PedNet and RODIN Study Group*

NEJM May 2013 Vol 368
Gouw et al.
NEJM May 2013 Vol 368

- 574 consecutive severe HA PUPs
- 75 exposure day follow up
- Cumulative inhibitor incidence 32.4%
- High titer inhibitor incidence 22.4%
1.6-fold increased inhibitor risk with 2nd Generation F-L rFVIII
# Recombinant FVIII Comparative Immunogenicity

<table>
<thead>
<tr>
<th>All inhibitors</th>
<th>RR$^*$</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>RODIN</td>
<td>1.60**</td>
<td>(1.08 - 2.37)</td>
</tr>
<tr>
<td>FCN</td>
<td>1.55**</td>
<td>(0.97 - 2.49)</td>
</tr>
<tr>
<td>UKHCDO</td>
<td>1.64**</td>
<td>(0.94 - 2.87)</td>
</tr>
<tr>
<td>EUHASS</td>
<td>0.99</td>
<td>(0.62 - 1.61)</td>
</tr>
</tbody>
</table>

$^*$: 2$^{\text{nd}}$ vs 3$^{\text{rd}}$ generation

$^{**}$: adjusted for major confounding variables

No difference in inhibitor risk between rFVIII vs p-dFVIII

Gouw et al
NEJM
January 2013
A Randomized Trial of Factor VIII and Neutralizing Antibodies in Hemophilia A

1st prospective randomized controlled study of FVIII inhibitor incidence

1.87-fold increased Hazard Ratio with recombinant FVIII products

44.5%

26.8%

No. at Risk
Recombinant factor VIII 126 105 80 70 60 52 50 44 41 41 40
Plasma-derived factor VIII 125 113 95 84 79 67 59 55 54 51 50

General Comments on a Biological Explanation for Differential Immunogenicity

Given –

1. Length of time to suggest a difference in immunogenicity (>25 yrs)
2. Immunogenicity difference (1.6 to 1.87-fold), is not binary (yes/no)

Biological Explanation will likely be -

- Relatively subtle
- Complex
Why Might Recombinant FVIII be More Immunogenic?

• Increased protein aggregates

• Different post-translational modifications
  ➢ eg, different glycans, sulfation

• Lacking supplemental plasma immunodulatory proteins
  ➢ eg. TGFβ, IL-10 etc.

• Possible immunomodulatory role for infused VWF
  ➢ ?quantity, ?heterogeneity of VWF
Innate Immunity

Antigen Presenting Cell

Adaptive Immunity

Stages in the FVIII Immune Response

CD4+ T Helper Cell

B Cell

Plasma Cell

Antibodies
Co-Localization of FVIII with Splenic Marginal Zone Macrophages (MARCO +ve)

Human rFVIII Infusion into Hemophilia A Mouse

Jesse Lai – work in progress
Innate Immunity ++

Adaptive Immunity ++

Antigen Presenting Cell

CD4+ T Helper Cell

B Cell

Plasma Cell

Recruitment ++

Antibodies

Innate Immunity ++

Adaptive Immunity ++
The ‘Danger Theory’ and FVIII inhibitor development

Splenic Antigen Presenting Cell

Safe!

No maturation
↑ T-cell tolerance

Danger!

↑ Maturation
↑ Co-stimulatory molecules
↑ Immunogenicity

APC

Status

PAMPs: TLR ligands (bacteria/virus)
DAMPs: HSP, histones, ATP, DNA
Early Events in FVIII Exposure to the Immune System
Product-Related Factors Likely to Influence FVIII Immunogenicity in PUPs
Laboratory Testing for the FVIII Immune Response

Anti-FVIII antibody generation (IgG₄/IgG₁)

Proceedings: A more uniform measurement of factor VIII inhibitors


The Bethesda Assay

114 Centers
Median Inhibitor: 5 BU
Range: 0-64 BU
CV 106%
(39% with 1 outlier removed)

Jennings et al. Haemophilia 2009
Are there better laboratory tests to detect the anti-FVIII immune response?
Are there better laboratory tests to detect the anti-FVIII immune response?

There Must Be!!!!!!!!!!
Presence of high affinity IgG in inhibitor patients

A

HA-INH (n=24/24)

B

HA-noINH (n=4/77)

High affinity anti-FVIII IgG in acquired hemophilia

Time course of the early anti-FVIII immune response

Conclusions

1. FVIII immune responses remain the most serious complication of hemophilia A therapy.

2. There is increasing evidence that the immune response to rFVIII is different to that for pdFVIII.

3. FVIII inhibitor mitigation requires more knowledge concerning the initial exposure of the immune system to FVIII.

4. Variations of the Bethesda assay are critical for clinical management but the assay is impossible to standardize.

5. Additional immunological assays may provide benefits for detecting the early phase of inhibitor development.