Hemophilia Therapy

From Rasputin to Recombinants

Hemophilia etiology, pathology and treatment

Hemophilia in history
Alexis and Rasputin
Advances 1920–70
AIDs and recombinants
Future therapy
Hemophilia

- Anatomic bleeding caused by congenital single-factor deficiencies
- 85% factor VIII deficiency (hemophilia A)
  - 1 in 10,000 male births
- 14% factor IX deficiency
  - Hemophilia B or Christmas disease
  - 1 in 30,000 male births
- 1% XI (autosomal, Rosenthal syndrome)
- Rare autosomal recessive single factor deficiencies
  - Prothrombin, V, VII, X, XIII

Hemophilia A Inheritance

- Sex-linked recessive, 1/10,000–20,000
- 25–30% spontaneous mutations
Hemophilia A Symptoms
Spontaneous anatomic (soft-tissue) bleeds

- Bleeding at umbilical stump, circumcision
- Delayed bleeding following injury
  - Joints, muscles, body cavities, GI, soft tissue, tongue, kidney, testicles, CNS
- Often spontaneous, especially joints
- Inflammation, hematomas, hemarthroses

<table>
<thead>
<tr>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td>15%</td>
<td>70%</td>
</tr>
<tr>
<td>6-30% VIII</td>
<td>1-5% VIII</td>
<td>&lt;1% VIII</td>
</tr>
<tr>
<td>Bleed after major trauma</td>
<td>Bleed after minor trauma</td>
<td>Spontaneous bleeding</td>
</tr>
</tbody>
</table>

Hemorrhages
Other Complications

- Lifestyle
- Economic
- Vocational
- Neurologic
- Psychological
- Lack of insurance
- Narcotics addictions
- Social: Russian revolution, for example

Hemophilia A Genetics

- 186 kb gene on X chromosome
  - Deletions, stop codons, missense and nonsense point mutations
  - 25–30% spontaneous mutation rate
  - Predominantly quantitative deficiency
  - Male hemizygotes are affected
- Hemophilia A in females is rare
  - Acquired anti-coagulation factor VIII
  - Autosomal hemophilia or VWD type N (Normandy)
  - Random excess Lyonization
  - Hemophilic father, carrier mother
The Fritsma Factor

Factor VIII Glycoprotein Cofactor

- 285,000-D heterodimer
  - Translated from the X chromosome
- Cleaved by thrombin, leaving a Ca^{++}-dependent portion that detaches from VWF and binds factor IXa and phospholipid
- Stabilizes IXa in the “tenase” reaction
- Deficiency slows thrombin production
- In vitro, deteriorates 5%/h at 18–24°C

VIII/VWF Interaction with Platelets

VWF binding site
GP Ib/IX/V
CD 42a-d

PLT

VIII:C

VWF

Internal Elastic Lamina

SMC: Smooth muscle cell
FB: Fibroblast
Lines: Collagen

SMC

THE FRITSMFACTOR
Your Interactive Hemostasis Resource

The Fritsma Factor
The Fritsma Factor

PT and PTT Test Results in Inherited Coagulopathies

<table>
<thead>
<tr>
<th></th>
<th>PT</th>
<th>PTT</th>
<th>Single Factor Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long</td>
<td>Long</td>
<td>Normal</td>
<td>VII</td>
</tr>
<tr>
<td>Long</td>
<td>Long</td>
<td>&quot;X, V, II, and fibrinogen&quot;¹</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>Long</td>
<td>&quot;VIII, IX, XI&quot;²</td>
<td></td>
</tr>
</tbody>
</table>

¹PT and PTT prolong only when fibrinogen is < 100 mg/dL.

²Contact factor deficiencies (XII, PK, HMWK) also prolong PTT, but do not cause bleeding.
Factor VIII, IX, or XI Assay

- Dilute plasma 1:10, add factor-depleted reagent plasma 1:1
- Add PTT reagent, incubate 3 minutes
- Add CaCl$_2$, record interval to clot formation
- Compare result in seconds to dilution curve

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Factor VIII Assay Dilutions
Parallelism Indicates No Inhibitor

<table>
<thead>
<tr>
<th>Plasma Dilution</th>
<th>Seconds</th>
<th>Raw Factor VIII Activity</th>
<th>Computed Factor VIII Activity (x dilution)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 (undiluted)</td>
<td>90 s</td>
<td>20%</td>
<td>20%</td>
</tr>
<tr>
<td>1:20</td>
<td>104 s</td>
<td>10%</td>
<td>20% (parallel)*</td>
</tr>
<tr>
<td>1:40</td>
<td>107 s</td>
<td>5%</td>
<td>20% (parallel)</td>
</tr>
<tr>
<td>1:80</td>
<td>110 s</td>
<td>2.5%</td>
<td>20% (parallel)</td>
</tr>
</tbody>
</table>

* < 10% difference from undiluted implies parallelism;
> 10% difference from undiluted = non-parallel, implies inhibitor
Factor VIII, IX, XI Assays at Four Dilutions: Lupus Anticoagulant

<table>
<thead>
<tr>
<th>Plasma Dilution</th>
<th>F VIII</th>
<th>F IX</th>
<th>F XI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 (undiluted)</td>
<td>17 %</td>
<td>20 %</td>
<td>5 %</td>
</tr>
<tr>
<td>1:20</td>
<td>26 %</td>
<td>22 %</td>
<td>6 %</td>
</tr>
<tr>
<td>1:50</td>
<td>50 %</td>
<td>30 %</td>
<td>12 %</td>
</tr>
<tr>
<td>1:100</td>
<td>74 %</td>
<td>32 %</td>
<td>17 %</td>
</tr>
</tbody>
</table>

- 10% change between dilutions = non-parallel
- Proceed to lupus anticoagulant and anti-phospholipid antibody profiles


Hemophilia A Therapy

- Monoclonally purified VIII concentrates available from many distributors
  - (Monarch M, Monoclate-P, Hemofil M)
- Recombinant factor VIII concentrates for PUPs (Recombinate, Kogenate)
  - Also B-domain-deleted factor VIII
Calculating Factor Dosage for Hemophilia A, B, or VWD

- One unit of factor = amount of activity in 1 mL normal plasma, same as 100%
- Determine plasma volume:
  - Blood volume (mL) = weight (kg) x 70 mL/kg
    - Use 60 for obese, BMI 25-30, 50 for BMI > 30
  - Plasma volume (mL) = blood volume (mL) x (100% - HCT%)
- Determine units of factor required:
  - Units of factor required = (desired factor level in units/mL - initial units/mL) x plasma volume (mL)

Example Factor Dosage for Factor VIII, IX, or VWF

- Patient: 80 kg, HCT 40%, 0 factor level
- Determine plasma volume:
  - Blood volume (mL) = 80 kg x 70 mL/kg = 5600 mL
  - Plasma volume (mL) = blood volume (5600 mL) x (100% - 40% = 60%) = 3360 mL
- Determine units of factor required:
  - Wish to reach 80% factor level (0.8 U), therefore…
  - Units of factor required = (0.8 U/mL - 0) x 3360 mL = 2688 (2700) U
  - Typical factor vial provides 1000 U
- Apply formula to conserve therapeutics
If Factor Assay not Available

- When factor assay not available and timing is critical, assume 0 activity or…
- The PTT may be correlated to factor levels

<table>
<thead>
<tr>
<th>Factor</th>
<th>PTT</th>
</tr>
</thead>
<tbody>
<tr>
<td>40%</td>
<td>35 s</td>
</tr>
<tr>
<td>30%</td>
<td>50 s</td>
</tr>
<tr>
<td>20%</td>
<td>65 s</td>
</tr>
<tr>
<td>10%</td>
<td>90 s</td>
</tr>
<tr>
<td>1 %</td>
<td>120 s</td>
</tr>
</tbody>
</table>

- Draw specimen and freeze, assay later
- Maintain patient database

Hemophilia A & B Inhibitors

- Assay factor VIII or IX
  - 30% of treated boys
  - Some dose and severity response
  - If > 30% factor VIII, no inhibitor is present
  - 3% of factor IX deficiencies
- Perform Bethesda titer
  - Reciprocal of patient titer that neutralizes 50% of factor VIII or IX in normal plasma
Factor VIII Assay Dilutions

non-Parallelism Indicates Inhibitor

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<th>Raw Factor VIII Activity</th>
<th>Computed Factor VIII Activity (x dilution)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:10 (undiluted)</td>
<td>80 s</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>1:20</td>
<td>93 s</td>
<td>8%</td>
<td>16%</td>
</tr>
<tr>
<td>1:40</td>
<td>107 s</td>
<td>5%</td>
<td>20%</td>
</tr>
<tr>
<td>1:80</td>
<td>108 s</td>
<td>4%</td>
<td>32%</td>
</tr>
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Factor VIII Inhibitor Therapy

- Factor IX complex, activated prothrombin complex concentrate (PCC), prepared by extraction
  - FEIBA, Autoplex are activated PCCs
  - Thrombosis (DIC) potential
- FEIBA dosage
  - 50 U/kg/12 h standard
  - 70 U/kg/8 h hemorrhage
  - Limit 200 U/kg/24 h to avoid DIC
  - Cannot monitor in laboratory
Recombinant VIIa Concentrate

- Effective dosage: 90 µg/kg
- Cannot monitor in laboratory
- Repeat full dosage every 3–6 h
  - 6h factor VII half-life
- $0.83/µg
- For a 75 kg patient, one dosage = $5600

1st Documented Bleeder’s Disease

2nd century: Talmudic ruling of Rabbi Judah the Patriarch exempts a woman’s 3rd son from circumcision if two elder brothers had died of bleeding after circumcision

2nd century: Rabbi Simon ben Gamaliel forbade a boy to be circumcised after sons of his mother’s three elder sisters had died after circumcision

11th century: Arabic surgeon Albucasis describes village males who bled to death from “trivial” wounds

1791–1803: British & American Families

- 1000–1800: Several references to “bleeders”
- 1791 (Britain), Zoll: 6 brothers bled to death after minor injuries
  - Half-siblings by a different mother were unaffected
- 1803 (Philadelphia), Otto: “A hemorrhagic disposition existing in certain families”
  - Recorded males in his own family with symptoms and recognized transmission through asymptomatic women
  - Traced pedigree to a woman named Smith in Plymouth, 1720–30


1800–28: Documented Names

- Bleeding disease
- Haemorrhoea
- Idiosyncrasia haemorrhagica
- Hereditary haemorrhagic diathesis
- 1828: first use of "haemophilia" (blood-loving) appears in the title of a treatise by Hopff at University of Zurich

Buloch and Fildes

Buloch W, Fildes P. *Treasury of human inheritance, parts V and VI, section XIVa, haemophilia.*

Published as Eugenics Laboratory memoirs XII, Francis Galton Laboratory for National Eugenics, University of London; 1911, Dulau and Co, 37 Soho Square, London.

- 1000 references and case reports
- 200 pedigrees
- Identified haemophilia as sex-linked, but carrier status not understood
- Meticulously traces the current spread of the mutation throughout Queen Victoria’s family

Alexandrina Victoria; May 24, 1819–Jan 22 1901, was *Queen of the United Kingdom of Great Britain and Ireland* from June 20, 1837 until her death, altogether 63 years and 7 months. The Victorian era was a time of UK industrial, political, imperial, and military progress.
Queen Victoria

• Presumed spermatogenesis mutation in father; Edward, Duke of Kent, who was in his 50s when Victoria was conceived
• Victoria’s seventh child, Leopold, was hemophilic
  – Stigmatized as a “weak” invalid by his mother
  – Married at 29
  – Died of cerebral hemorrhage following a fall at 31
• Two daughters, Alice (2nd) and Beatrice (8th) were carriers

Victoria Eugenie (Ena) Battenberg

- Beatrice’s daughter, 2 hemophilic brothers
- Alfonso XIII of Spain married her in 1906
  - Warned by Spanish Embassy, but trusted his luck
- Alfonso XIV b. 1907 bled from circumcision
  - Alfonso blamed Ena, grew to despise her
  - Jaime, deaf-mute; Gonzalo, hemophilic
  - Spaniards blamed the British
- Juan b. 1913, normal
  - Son Juan Carlos is current King
- 1931, royal family in exile in Rome
  - Alfonso was too ill to appear
  - 1933, marries Cuban commoner and abdicates
The Romanovs: Tsar Nicholas II, Alexandra (Alix, granddaughter of Victoria) and Family

Prince Alexis, 1912
The Fritsma Factor

Grigori Yefimovich Rasputin
1869–1916

Rasputin

- 1869, Pokrovskoye, Siberia
- Two sibs drowned
- 1887: three months in Verkhoturye Monastery
- 1901: strannik (pilgrim) wandered through Greece, Jerusalem
- 1903: Saint Petersburg, starets (holy man) with healing & prophetic powers
- 1905: Alexandra introduced by Anna Vrubova to get help for 1 YO Alexis
Rasputin’s Power: 1905–16

- Calming influence?
  - 1912 in Spala, Poland “Don’t let the doctors bother him too much; let him rest” (by telegram)
  - Distraction, causing Alexis to relax?
- Faith healer, hypnotism?
- Leeches?
- Aspirin?

Alexandra believed God spoke through Rasputin, and he became the czar’s primary adviser and gatekeeper, used his power for financial gain and debauchery, and was increasingly hated by the Russian nobles, though loved as a prophet by many of the peasants.

The Romanovs in 1912

Pierre Gilliard, Alexis’ tutor, wrote: “The illness of the Tsarevich cast its shadow over the whole of the concluding period of Tsar Nicholas II’s reign. Without appearing to be, it was one of the main causes of his fall, for it made possible the phenomenon of Rasputin and resulted in the fatal isolation of the sovereigns who lived in a world apart, wholly absorbed in a tragic anxiety which had to be concealed from all eyes.”

Historians have since disputed the contribution of Alexis’ hemophilia to Russian politics, but the strain on the royal household is clear enough.
Rasputin and Admirers, 1914

Yusupov Moika Palace, St. Petersburg
Maj. Felix Yusupov: Dec 16, 1916

Poisoning Rasputin

Cyanide-laced cakes
Treatment Attempts 1901-1942

- Lime
- Gelatin
- Oxygen
- Splenectomy
- Bone marrow
- Sodium citrate
- Calcium lactate
- Witte’s peptone
- Hydrogen peroxide
- Induced anaphylaxis
- Antidiphtheric serum
- The ‘galvanic needle’
- Animal and human sera
- Adrenaline
- Bird's muscle
- IV oxalic acid
- Vitamin therapy
- X-ray irradiation
- Serum from the mother
- Tissue fibrinogen by mouth
- Bromide extract of egg white; sedative
- Blood—both injected and withdrawn therapeutically, autohemotherapy
- Female hormone therapy (in the belief that femininity prevents the expression of the hemophilic gene)

Effective Treatments

- 1926, Surgeon General: 12 referenced attempts at whole blood transfusion
- 1934, McFarlane: topical application of Russell viper venom
- 1937, Patek and Taylor first characterization of anti-hemophilic globulin
- 1938, McFarlane: fresh whole plasma
- 1950s: EJ Cohn fractionation of whole human and animal plasma
  - Kekwick and Wolf, 1957; Soulier, Gobbi, Larrieu, 1957; Blomback, Blomback, Nilsson, 1958
1952: Stephen Christmas
Canadian Hemophilia Society:
Delineation of factor IX deficiency
(Christms disease) from factor VIII deficiency

1953

Breakthroughs

• 1964, Judith G. Pool (1919–75, U of Chi)
  – Cryoprecipitate
  – First opportunity for hemophilic home care

• 1968, Kenneth M. Brinkhous (1908–2000, UNC Chapel Hill)
  – First to chemically characterize factor VIII in 1938
  – Developed AHF with hemophilic dog experiments
  – AHF released through Hyland in 1968
Advances in the 1960–70s

- Dental extractions and minor procedures using Kohn fractions
- Orthopedic correction of hemarthroses using AHF
- Prothrombin complex concentrate, II, VII, IX, X for inhibitor
- Home therapy
- Hemophilia treatment centers 1973
- By 1980, life expectancy was 60
- But high hepatitis rate, 20,000 donors/pool
  - No viral inactivation

AIDS

- Fall 1980: *Pneumocystis carinii* pneumonia and Kaposi sarcoma in homosexual males
  - Searched for non-infectious causes such as amyl nitrite “poppers,” anti-sperm antibodies or anal intercourse
- Spring 1982: CDC recorded three cases of PCP in hemophiliacs receiving AHF, all died
  - Reports of similar symptoms in Haitian hemophiliacs and drug abusers
  - No homosexual behavior or illegal drug use
  - Led to concept of blood-borne viral infection

July 27, 1982

- CDC reported to blood and plasma industries, gay organizers, hemophilia organizations, FDA and NIH
- Consensus to not act
  - Evidence of three patients too weak to conclude hemophils were a risk group
  - Concern it would stigmatize homosexual community
  - Lose homosexual donors to blood and plasma services
  - Millions of dollars to change manufacturing policies
- Fall of 1982: six more cases, including two non-hemophilic blood recipients
  - Donor services would not reveal donor identities to avoid embarrassing and subsequently losing donors
  - One definite identification

January 4, 1983

- CDC reported the statistical prevalence of hepatitis B was identical in hemophils and AIDS risk groups (surrogate association)
- CDC reported to the same groups including ARC, AABB, National Hemophilia Foundation, National Gay Task Force, Pharmaceutical Mfrs Association, Council of Community Blood Centers, State and Territorial Epidemiologists, and individuals.
- Again, consensus to not act, debate was irrational, acrimonious and public, harshly critical of BL Evatt and CDC
January 13, 1983

- CCBC and AABB: “transfusions are life-saving procedures; some adverse reactions are acceptable to save lives. The rare disorder affecting nine cases is not enough to force a policy change.”
- ARC head Dr Cumming wrote: “It has long been noted that CDC increasingly needs a major epidemic to justify its existence… In short, we can not depend on the CDC to provide scientific, objective, unbiased leadership.”
- NHF, however, already alarmed, had contacted plasma manufacturers in December, 1982

NHF Initiative: 1983

- Dec 1982: Alpha Therapeutics began to screen donors
- 20% of commercial plasma came from donor services who refused to screen donors for sexual orientation
- US Public Health Service guidelines, March 4, 1983
  - CDC bypassed FDA, sent guidelines direct to PHS
  - Donor screening and surrogate testing: hepatitis markers
- March, 1983, Baxter Hyland began heat treating plasma
- August, 1983, 26 confirmed cases of transfusion transmitted AIDS, including one F IX deficiency
Virus Isolation: 1983

- Mid-1983: Pasteur Institute isolates virus from lymphadenopathy patients
- Feb, 1984: Pasteur Institute isolates virus from AIDS samples provided by CDC
- Sep, 1984: Alpha and Cutter demonstrate heat treatment is safe and does not increase immunogenicity
- Oct, 1984: CDC/PHS screening and heat treatment guidelines published and adopted
- By 1984, 63% of 15,500 US hemophilia patients had HIV
- Since 1/1/1985, not a single new factor-transmitted HIV infection has been recorded

Recombinant Clotting Factors

- 4/7/1976: Genentech incorporated
- 1981: Genetics Institute incorporated
- 1982: rFIX cloned by both (small molecule)
- Aug, 1984: Both cloned rFVIII gene and produced the protein
- 1985: rVWF coexpressed
rFVIII Barriers

- Extensive post-translational modification
  - Need for mammalian cloning system
  - Chinese hamster ovary cells
- rFVIII affinity for phospholipid
  - CHO membrane binds and degrades FVIII
  - CHO cells transfected with FVIII and VWF genes
  - Resolved by rVWF coexpression—higher affinity
- Need for serum-free cloning system
  - Eliminate animal viruses, ease purification
- High-volume fermentation process
  - Immunoaffinity, ion-exchange, pasteurization, solvent-detergent, nanofiltration reduces 7000 L to 1 L.

rFVIII to Market

- Regulatory uncertainty
  - Sterility, consistency questions generate 600 tests/lot
- Baxter Hyland and Genetics Institute produced Recombinate®
- Bayer Miles Lab/Cutter Biological and Genentech produced Kogenate®
- 3/27/1987: UNC Chapel Hill, first phase II infusion into a 39 year-old hemophiliac
- Recombinate® cleared 1992, Kogenate® 1993
**Post-market Advances**

- B-domain deleted: ReFacto®
- Removal of human and animal protein additives (albumin): Advate®
- Hemostatic efficacy: 90% cessation of bleeding
- Risk of inhibitor formation in previously untreated persons (PUPs) is approximately double plasma-derived FVIII (pdFVIII)
- Risk of inhibitor formation in PTPs < 1%


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**rFIX**

- 1998: Genetics Institute BeneFix®
- Barrier: γ-carboxylation of 12 glutamic acids at amino terminus
  - Vitamin K
  - Coexpressed γ-glutamyl carboxylase
  - Coexpression of furin
- 90% bleeding cessation using 1–2 infusions
- PUPs—3% high titer inhibitors
1999: rFVIIa
- Same γ-carboxylation issue
- For inhibitors: generates no DIC compared to activated prothrombin complex concentrates
- Activates through tissue factor and platelet surface binding
- Activates thrombin activatable fibrinolysis inhibitor (TAFI) to control fibrinolysis
- Novel variants in animal models

Primary Prophylaxis in Children
- 2005: Joint damage outcome study
  - 25 IU/kg every other day generates 6X decrease in joint deterioration by MRI vs on-demand (OD) Rx up to 6 YO
- 2009 Italian study on prophylaxis
  - 10 Y f/u on 25 IU/kg 3X a week showed 0.52 vs 1.08 total bleeds and 0.2 vs 0.52 joint bleeds/patients/month in OD
- 2009 Danish/Russian study
  - Orthopedic issues 15.6 in OD vs 2.2 in prophylaxis

Primary Prophylaxis in Children

- **Encouragement**
  - Abundant safe rFVIII

- **Barriers**
  - Venous access with clotting, cost, compliance, duration

- **Inhibitor formation in severe hemophilics**
  - Meta-analysis of 20 trials
  - 1248 patients, 798 severe, 632 treated exclusively with plasma-derived and 616 with recombinant FVIII, median age 12
  - Combined trials data: 14% inhibitors in PD FVIII, 25% in rFVIII
  - High titer: 8.8% PD, 12.3% recombinant


Future

- rVWF
- rXIII
- **Gene transfer**
  - One human trial was negative
  - Animal trials in progress
- **Need for new bioassays**
  - Chromogenic FVIII
  - Activated FVII
- **Need for humane public policies**