Hemophilia: Rasputin to Recombinants

The Fritsma Factor
Your Interactive Hemostasis Resource

Hemophilia Therapy
From Rasputin to Recombinants

- 40 YO Af-Am Hemophilic
  - Bleeding into ankle
    - Anatomic soft-tissue and joint bleeds
    - Tried RICE, DDAVP inhaler
  - Ran out of factor VIII (FVIII) concentrate
    - Provided free by Medicare through hemophilia center
    - National Hemophilia Foundation: www.hemophilia.org
  - Clinical path resident on-call; night tech
    - Determine patient residual factor VIII activity
    - Order antihemophilic factor (AHF) VIII concentrate
      • Dispensed by transfusion service
    - Compute and prepare dosage
    - Reconstitute with sterile water, administer as IV push
    - Subsequently determine therapeutic factor VIII activity

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 bleeds, 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>

Other Complications

- Lifestyle
- Economic
- Vocational
- Neurologic
- Psychological
- Lack of insurance
- Narcotics addictions

Cerebral hemorrhage

Hemarthroses
Airway Obstruction

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

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Calculating AHF Dosage

• One unit of factor = amount of activity in 1 mL normal plasma, same as 100%
• Determine plasma volume based on weight
  – Blood volume (BV, mL) = weight (kg) x 70 mL/kg
    • Use 60 mL/kg for obese, BMI 25–30
    • Use 50 mL/kg for morbidly obese, BMI > 30
  – Plasma volume (PV, mL) = BV x (100%−HCT%)
• Determine units of AHF required
  – Units of AHF required = \[ (\text{desired AHF in units/mL} - \text{initial units/mL}) \times \text{PV (mL)} \]

40 YO Af-Am Hemophilic

• 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) \times (100%−40%) = 60% \times 5600 = 3360 mL
• Determine units of factor required:
  – Wish to reach 80% factor level (0.8 U/mL), therefore…
  – Units of factor required = \( (0.8 \text{ U/mL} - 0) \times 3360 \text{ mL} = 2688 \text{ (2700) U} \)
  – Typical FVIII concentrate vial provides ~1000 U, use 3
  – Avoid overdose: thrombotic and wasteful

If Factor Assay not Available

• When factor assay not available and timing is critical, assume 0 activity or…
• Approximate factor VIII from PTT

<table>
<thead>
<tr>
<th>Factor VIII</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>

• Collect baseline plasma
  – Assay or freeze and confirm with assay next day shift
• Maintain patient database

Plasma-derived FVIII Concentrates

• Risk of HBV, HCV, HIV transmission
  – Human and animal serum
  – Hemofil-M®, Monarc-M®, Monoclate-P®
  – Purification: immunoaffinity column, solvent-detergent, Pasteurization, viral filtration, combinations
  – $0.35–0.60/U
• Seroconversion per CDC surveillance: 0
  – Predicted risk, 1:60,000

Recombinant FVIII Concentrates

• Serum in culture medium
  – Helikate®, Kogenate®, Recombinate®
  – $0.68–1.05/U: some select for only previously untreated patients (PUPs)
  – No HBV, HCV, HIV seroconversions
• No protein in culture or prep
  – Calculated viral risk=0, actual=0: Advate®
• B-domain-deleted FVIII concentrate
  – Human albumin: ReFacto®, Nonacor®
  – No protein: Xyntha®
  – Can’t assay using clot-based factor VIII assay, use chromogenic

Factor VIII Assay

• Dilute plasma 1:10, add factor VIII-depleted reagent plasma 1:1
• Add PTT reagent, incubate 3 minutes
• Add CaCl₂, record interval to clot formation
• Compare result in seconds to dilution curve
### 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 &quot;undiluted&quot;</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 indicates parallelism, no inhibitor*

### 40 YO Af-Am, FVIII Inhibitor

- **Inhibitor:** alloantibody to FVIII concentrate
  - 30% incidence
  - Dose and severity response: mostly severe hemophilia
- **Factor VIII assay**
  - If non-parallel, reflex to Bethesda titer
- **The "poor man’s" Bethesda titer substitute**
  - Dilute plasma 1:20 in normal plasma
  - If prolonged, presume >5 BU, treat as high titer
    - Corticosteroids and FEIBA® or NovoSeven® (VIIa)
  - If not prolonged, presume <5 BU, treat as low titer
    - Factor VIII concentrate
  - Confirm with full Bethesda titer
    - Larry D. Brace, PhD, Edward Hospital, Naperville, IL

### 40 YO Af-Am, FVIII Concentrate Therapy

- **Peak:** 15 m after administration: 0.3 U/mL
  - Should have been 0.8, what happened?
  - Suspect anti-factor VIII inhibitor
  - If peak reaches expected value, go on to next administration
- **Nadir:** 12 h after administration
  - Reflects half-life, should reach 50% of desired activity
  - Administer new AHF, use half the dosage second time

### FVIII Assay Dilutions

**Non-Parallelism Indicates 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 &quot;undiluted&quot;</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>
</tbody>
</table>


### FVIII Inhibitor Therapy

- **Plasma-derived factor IX complex**
  - Activated prothrombin complex concentrate (PCC)
  - Prepared by BaSO₄ extraction
  - Available since 1980
  - FEIBA®, Autoplex®
- **FEIBA dosage**
  - 50 Units/kg/12 h standard
  - 70 Units/kg/8 h in hemorrhage
  - Limit 200 U/kg/24 h to avoid DIC risk: fatal
  - Cannot monitor: only general evaluation with PTT

### Recombinant FVIIa Concentrate

- **Dose:** 90 μg/kg
  - Repeat every 3–6 h
  - 6 h FVII half-life
- **$0.83/μg**
  - For our 80-kg patient, one dose = ~$6000
- **Cannot monitor**
  - General evaluation using PTT
  - No risk of DIC
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 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

Factor VIII is a 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
The Fritsma Factor

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 unafflicted
• 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


Bulloch and Fildes

Bulloch 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

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) turned out to be carriers


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.

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Queen Victoria and Family

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 XIII blamed Ena, grew to despise her
    - Jaime, deaf-mute; Gonzalo, hemophilic
    - Spaniards blamed the British
- Juan B. 1913, normal
- 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
- Alexis Nikolaevich Aug 12, 1904

Prince Alexis, 1912

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 Rises
1912 Belovezhski Forest Holiday

- Faith healer, hypnotism?
- Leeches?
- Calming influence, distraction?
  - ‘God has seen your tears and heard your prayers. Don’t be sad, the little boy is not going to die. Don’t let the doctors frighten him.’

Rasputin 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 mythical figure by some 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.

Yusupov Moika Palace, St. Petersburg

Maj. Felix Yusupov: Dec 16, 1916

Wax figures at the Yusupov palace
Poisoning Rasputin

- Cyanide-laced cakes

Treatment Attempts 1901–1942

- Lime
- Gelatin
- Oxygen
- Splenectomy
- Bone marrow
- Sodium citrate
- Calcium lactate
- Wilte'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, auto-hemotherapy
- 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

Breakthroughs

- 1964, Judith G. Pool (1919–75, U of Chi)
  - Developed 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


Rev. Robert Massie, Jr. Susan Massie

The Fritsma Factor

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 hemophils receiving AHF, all died
  - Reports of similar symptoms in Haitian hemophils and drug abusers
  - Led to concept of blood-borne viral infection


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 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

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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 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%

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 (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

Encouragement
• Abundant safe rFVIII

Barriers
• Venous access with clotting, cost, compliance, duration

Inhibitor formation in severe hemophiliacs
• 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 FVIII
• Need for humane public policies