PrecisionBioLogic

Introduction to Hemophilia

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Senior National Technical Sales Manager

CELP April 2019

CRYOcheck™ XXV YEARS | 25 years of leaving errors out in the cold.

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Learning Objectives

1. Describe the types of Hemophilia that effect patients.
2. Explain the range of hemophilia symptoms, along with diagnosis and common treatments.
3. Relate the methods and clinical application for mixing studies and Factor Inhibitors.

WFH WORLD FEDERATION OF HEMOPHILIA

WORLD THROMBOSIS DAY 13 OCTOBER

The Fritsma Factor

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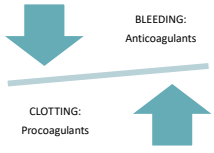
Hemostasis

A delicate balance

Complex process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere

When out of balance, hemorrhage or thrombosis can be life-threatening



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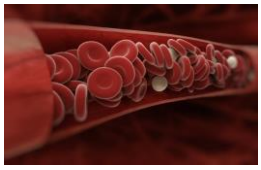
Hemostasis

Two main phases

Primary hemostasis

Secondary hemostasis



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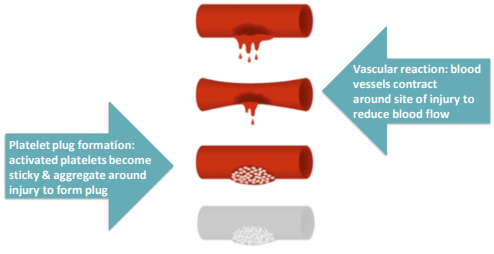
Primary Hemostasis

Vasoconstriction and platelet plug formation

Initial, rapid, short-lived response to vessel damage



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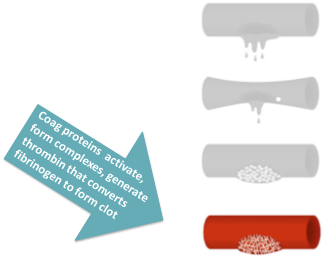
Secondary Hemostasis

Activation of coagulation proteins to form a stable fibrin clot

Followed by fibrinolysis, gradual digestion & removal of clot as healing occurs



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Coagulation Proteins

A co-dependant group of serine proteases (enzymes) known as "factors"

These factors work together in a pro-coagulant manner to form a clot which will stop bleeding

Factors are typically inactive in circulation until activated

Once activated they form a "cascade", activating each other until a clot is formed

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Coagulation Proteins

Each factor belongs to a specific pathway

The Intrinsic and Extrinsic pathways are activated in different ways

Both pathways converge at a "Common" pathway where the coagulation process accelerates into fibrin clot formation

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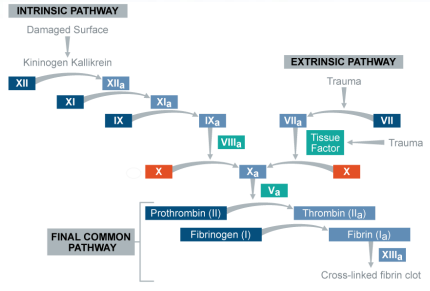
Coagulation Cascade



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Bleeding Disorders

Hemorrhage

Severe bleeding requiring physical intervention

May be localized or generalized, acquired or congenital

Localized (from a single location) commonly indicates injury, infection, tumor or isolated blood vessel defect

Generalized (from multiple sites, spontaneous/recurring, or requiring medical attention) may indicate defect or disorder and warrant hemostasis laboratory testing

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Bleeding Disorders

Congenital vs. Acquired

Congenital are:

- Diagnosed early in life
- Uncommon (<1 in 100 people)

Likely **acquired** if patient's bleeding episodes:

- Began after childhood
- Are associated with disease or trauma
- Not present in relatives

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Bleeding Disorders

Congenital

Most common **congenital**:

- Hemophilia A & B (FVIII & FIX deficiencies)
- von Willebrand disease (VWD)
- Hemophilia C (FXI deficiency)
- Platelet function disorders

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Bleeding Disorders

Screening Tests

Prothrombin Time (PT)

Prolonged clotting time may be indicative of a factor deficiency in the Extrinsic and common pathways

Activated Partial Thromboplastin time (APTT)

Prolonged clotting time may be indicative of a factor deficiency in the Intrinsic and common pathways

Thrombin Time / Fibrinogen

Assesses potential for fibrinogen abnormalities

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Bleeding Disorders

Confirmatory Tests

Mixing Studies

When either or both the PT/PTT screening assays exceed upper limit of lab's defined reference range

Detect factor deficiencies, LAs and specific inhibitors

Factor Assays

Detect and measure coagulation factor deficiencies

Bethesda Titers

Detect and measure coagulation factor inhibitors

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What is Hemophilia?

X-linked congenital bleeding disorder

Those with hemophilia bleed for longer than normal

Quite rare

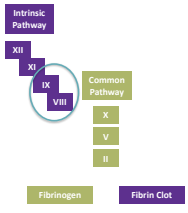
- Frequency: approx. 1 in 10,000 births
- Estimated 400,000 worldwide

Usually inherited

- About 30% of those with hemophilia have no family history (acquired hemophilia)

Two types

- Hemophilia A, FVIII deficiency
- Hemophilia B, FIX deficiency



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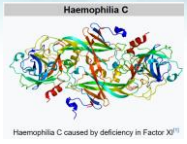
Hemophilia C

plasma thromboplastin antecedent (PTA) deficiency or **Rosenthal syndrome**



Haemophilia C caused by deficiency in Factor XI[1]

Factor XI Deficiency

Common among people of Jewish decent
Prevalence estimated at up to 3% of Ashkenazi Jews
Autosomal recessive-heterozygotes can bleed
Effects both males and females
Bleeding severity is not influenced by the level of factor XI
Mild form of hemophilia
Not prone to bleed spontaneously

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When Dinosaurs Still Walked

65 million years ago

The mutation giving rise to hemophilia occurs in at least three orders of placental mammals that existed at the end of the Cretaceous period

2nd Century AD

Rabbi Judah the Patriarch rules 3rd son exempt from circumcision if his two elder brothers died of bleeding after circumcision

Ingram GIC. The history of haemophilia. J Clin Pathol. 1976 Jun; 29(6): 469–479.

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Other Species

Figure 1
Irish Setter hemophilia A index case, University of North Carolina at Chapel Hill (photo by William Brinkhous).

Figure 2
Queen's University Hemophilia A dogs, early in existence of the colony. Pictures courtesy of Dr. David Lillicrap, Queen's University, Kingston, Ontario, CA.

Figure 3
Sheep with hemophilia A, depicting swollen and hemorrhagic knees from acute and chronic hemarthoses. Figure modified from Porada, CD, et al.[37]

Semin Hematol. 2013 Apr; 50(2): 175–184.
doi: 10.1053/j.seminhematol.2013.03.023

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Family Ties

1791

Obituary of Isaac Zoll, aged 19, the sixth brother to bleed to death following minor injuries; half-siblings born to a different mother unaffected

1820

Nasse's law: German physican C.F. Nasse defines the inheritance pattern

1828

Term Haemophilia (love of blood) is first used

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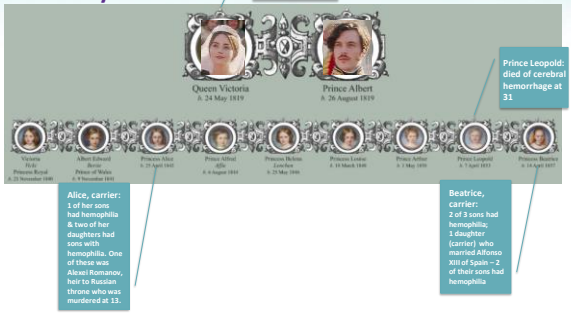
The Royal Disease



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Royal Disease

Tsarevitch Alexei Nikolaevich

Alexei was born on August 12, 1904. He was the youngest child and only son of Emperor Nicholas II and Empress Alexandra Feodorovna. Alexei was the heir to the throne of the Russian Empire. He was born with Hemophilia B (which could be traced back to his maternal great grandmother Queen Victoria). Alexei was killed with his parents and sisters during the Russian Civil War on July 17,1918.

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Famous Faces

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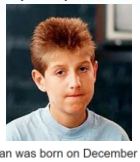
Famous Faces

Ryan Wayne White



Ryan was born on December 6, 1971 in Kokomo Indiana. He had prolonged bleeding after his circumcision. When he was 3 days old he was diagnosed with Severe Hemophilia A. He was getting weekly blood transfusions. In December 1984 he became very sick with pneumonia. The doctors then diagnosed him with AIDS and said he only had 6 months to live. Ryan got AIDS through blood transfusions that were not screened. He became famous because his school told him he was not allowed to attend and his parents filed a law suit through the U.S. District Court in Indiana. They eventually won the law suit. His law suit made national headlines and he ended up becoming the poster boy for AIDS. Despite what the doctors said, Ryan lived until April 8, 1990. His funeral was attended by 1,500 people including Elton John, Barbara Bush, Howie Long, Phil Donahue and Michael Jackson.

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Famous Faces

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Famous Faces

Richard was born November 10, 1925 in Wales. He died August 5, 1984 in Switzerland from a stroke. He was nominated seven times for an Academy Award , six of which were for Best actor in a leading role. He was in this business from 1944 -1984. Richard was in 61 films and 30 stage productions. He was married 5 times, twice to Elizabeth Taylor. Richard suffered from Hemophilia.

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Famous Faces-Rumored??

Abraham Lincoln

Abraham was born February 12, 1809. He became the 16th president of the United States. He successfully led the country through the American Civil War and ended slavery. He was shot in the back of the head on April 14, 1865. After 9 hours of being in a coma, he died at 7:22 am on April 15, 1865. Abraham is rumored to have had Hemophilia.

Genghis Khan

Genghis Khan was born around 1162 and died in August 1227. He was probably the most famous and brutal conquerer of all time. He was the founder of the Mongol Empire, which became the largest contiguous empire in history. Genghis was also rumored to have had Hemophilia.

Mother Teresa

Mother Teresa was born August 26, 1910 and died September 5, 1997. For over 45 years, she ministered to the poor, sick, orphaned, and dying, while guiding the Missionaries of Charity's expansion, first throughout India and then in other countries. She is rumored to have had Hemophilia.

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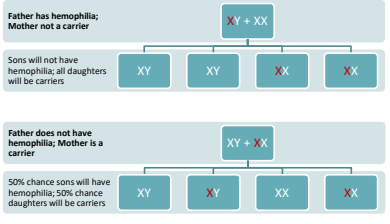
How is Hemophilia Inherited?

X-linked inheritance; males predominantly affected



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Severity of Hemophilia

Classified on plasma levels of FVIII or FIX activity

Three levels:

Level	% of normal factor activity	Occurrences
Normal range	50 – 150%	
Mild hemophilia	> 5 – < 40%	• Might bleed for long time after surgery/injury • Do not bleed without reason
Moderate hemophilia	1 – 5%	• Might bleed about 1x/month • Rarely bleed for no obvious reason
Severe hemophilia	< 1%	• Might bleed 1 or 2x/month • Might bleed for no obvious reason

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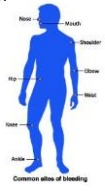
Symptoms

Bleeds can occur inside or outside the body; they may begin immediately, after a delay of several hours or spontaneously

- Large/unexplained bruises
- Bleeding into muscles and joints causing swelling, pain and stiffness
- Spontaneous internal bleeding for no obvious reason
- Prolonged bleeding after injury, dental work or surgery



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Hemophilia in Women

It's not just a male disease

- Regardless of gender, anyone with < 40% of the normal clotting factor has hemophilia
- Some carriers have symptoms even though their clotting factor levels are above 40%
- A woman with levels of 40-60% who experiences abnormal bleeding is called a symptomatic carrier
- In addition to the usual symptoms, symptomatic carriers and women with hemophilia might experience:
  - Heavy or prolonged menstrual bleeding
  - Postpartum bleeding
  - Other gynecological problems

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World Hemophilia Day 2019

April 17 is World Hemophilia Day –
a day to raise awareness about hemophilia and other inherited bleeding disorders
This year's focus is on reaching out and identifying new members of the bleeding disorders community

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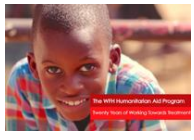
Hemophilia in Developing Countries

Lack of access to care and treatment is an urgent and important public health challenge due to the cost of products
Globally, 75% of people with bleeding disorders receive inadequate treatment or no treatment at all
Diagnosis is also a challenge
Organizations such as WFH are working to close the gap between the

- number of people born with hemophilia and those who reach adulthood
- estimated and actual number of people diagnosed
- amount of treatment product needed and what is available



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Complications

Physical complications
Frequent bleeds may result in debilitating and progressive musculoskeletal lesions and deformations
Neurological deficiencies after intracranial hemorrhage
Infection (drastically reduced since the introduction of sterilized and recombinant factor concentrates)
Psychological and economic complications
Stress, low self-esteem, depression
Limited productivity, time away from work/school
Development of inhibitors

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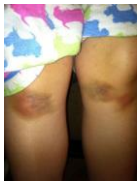
Diagnosis

Prenatal diagnosis can be done at 9-11 weeks by chorionic villus sampling (CVS) or by fetal blood sampling at 18 weeks or more

Newborns to a mother with family history of hemophilia are tested at birth

Severe hemophilia is usually diagnosed before the first year

Mild hemophilia may not be suspected until triggering event in late childhood or later



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Diagnosis

Accurate measure of activity is necessary to:

Make a diagnosis

Classify the severity

Monitor therapy

Clinical assessment: detailed bleeding and family history

Routine, screening tests

APTT *usually* prolonged

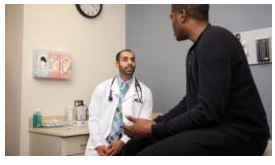
PT/INR is normal

TT is normal

Specialty coagulation tests

Mixing studies

Factor assays



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Hemophilia Treatment Centers (HTC's)

141 Federally designated HTC's across the US

Stanford University Medical Center
Division of Hematology/Oncology
1000 Welch Road, Suite 300
Mail Code 5798
Palo Alto, CA 94304

UCSF Benioff Children's Hospital Oakland
Division of Hematology/Oncology
747 52nd Street
Oakland, CA 94610-4131

University of California at Davis
Hemostasis and Thrombosis Center
UC Davis Medical Center
2000 Stockton Blvd., Suite 202
Sacramento, CA 95817

University of California San Francisco
Hemophilia Comprehensive Care Program
Pediatrics: 550 16th St., 4th floor, Mail Stop 0434, San Francisco, CA 94143
Adults: 350 Parnassus, Suite 407, San Francisco, CA, 94117
San Francisco, CA 94143-0434

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

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Mixing Studies

A first-line investigation

Uses normal pooled plasma mixed with patient plasma to either correct a factor deficiency or be influenced by an inhibitor in that patient plasma when using PT and/or APTT test system

Once differentiation is made, the lab can use algorithms leading to identification of deficient factor or type of inhibitor present

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Factor Assays

Diagnose or monitor treatment

Hemophilia A & B are commonly diagnosed through the use of a modified APTT assay

When a patient sample is mixed with FVIII/FIX deficient plasma, the degree of correction of the APTT is proportional to the level of FVIII/FIX in the patient plasma

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Factor Assays

One-stage clot assay

Based on APPT

Used by majority of clinical labs for all factor activity assays

Many instrument and reagent combinations available

Two-stage clot assay

Rarely performed: complex, cannot be automated, no kit available

Chromogenic assay

Based on two-stage clot assay

Limited availability in clinical labs, considered expensive, often performed as batched analysis

FVIII: multiple FDA-cleared kits; offered by few labs

FIX: no FDA-cleared kit, offered by few to no labs

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Management

Prevent bleeding

Avoid trauma such as:

- IM injections
- Arterial punctures
- Contact sports

Avoid antiplatelet agents and regular NSAIDs (e.g. aspirin)

Avoid herbal medicines suspected to cause bleeding (e.g. ginkgo biloba)

Replace missing factor prior to surgery and dental work

Patients, especially those with severe hemophilia, require regular prophylactic factor replacement therapy on a regular basis

Coordinate patient care with Hemophilia Treatment Center (HTC)

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Management

When to Treat?

If serious bleeding or trauma is suspected, treat first

Bleeding into a joint/muscle

Injury to neck, mouth, tongue, face or eye

Severe head blows and unusual headaches

Heavy/persistent bleeding

Severe pain or swelling

Open wounds requiring stitches

Rest, compression, elevation for affected muscles/joints

Follow therapy recommendations/consult Hematology or HTC for advice

Quick treatment helps:

- Reduce pain and recovery time
- Prevent damage to joints, muscles and organs
- Minimize the amount of blood product required to stop the bleeding

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Treatment Options

Clotting Factor Replacement Therapy (prophylaxis)

Factor replacement therapy	Definition
Episodic 'on demand' replacement therapy	Replacement therapy given at the time of clinically evident bleeding
Regular replacement therapy	Replacement therapy given to prevent bleeding
Primary prophylaxis	Regular continuous[a] replacement therapy started in the absence of documented joint disease, determined by physical examination and/or imaging studies, and before the second clinically evident joint bleed and age 3 years
Secondary prophylaxis	Regular continuous[a] replacement therapy started after two or more joint bleeds but before the onset of joint disease documented by physical examination and/or imaging studies
Tertiary prophylaxis	Regular continuous[a] replacement therapy started after the onset of joint disease documented by physical examination and plain radiographs of the affected joints
Intermittent 'periodic' prophylaxis	Replacement therapy given to prevent bleeding for periods not exceeding 45 weeks in a year

a Continuous is defined as the intent to treat for 52 weeks/year and receiving a minimum of an *a priori* defined frequency of infusions for at least 45 weeks (85%) of the year under consideration.

Blanchette VS, Key NS, Ljung LR, Manco-Johnson MJ, van Den Berg HM, Srivastava A, for the Subcommittee on Factor VIII, Factor IX and Rare Coagulation Disorders. Definitions in hemophilia: communication from the SSC of the ISTH. J Thromb Haemost 2014; 12: 1935–9.

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Factor replacement therapy

Calculation based on baseline level, desired level for clinical bleeding situation and rise in factor expected with replacement

Factor VIII replacement: each IU/kg results in 2% rise in factor activity; half-life of 8-12 hours

Factor IX replacement: each IU/kg results in 0.5-1% rise in factor activity; half-life of 18-24 hours

Situation	Desired Factor Level (IU/mL)	Dose of Recombinent FVIII (IU/kg)	Dose of Recombinent FIX (IU/kg)
Minor bleed	0.25-0.35	15-20	25-40
Moderate bleed/minor surgery	0.35-0.6	20-30	35-70
Severe bleed/major surgery	0.8-1.0	40-50	80-120

Bloody Easy: Coagulation Simplified, 2013, ORBCoN; 42.

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Treatment Products

Factor Concentrates

Plasma-derived

Widely used in the late 1960s & 1970s

Quality of life improved: home therapy, life-expectancy increased

By the early 1980s, however, epidemic of blood-borne viruses (HBV, HCV, HIV) transmitted by these concentrates

- By 1984, 63% of US hemophilia patients had HIV

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Treatment Products

Factor Concentrates con't

Efforts by patient advocacy groups & CDC resulted in donor screening and new manufacturing processes such as dry heat to kill viruses in plasma

CDC surveillance 1998-2002 reports no transmission

Safer treatments were sought

- Cloning of FIX gene in 1982 and FVIII gene in 1984 paved the way for recombinant products

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Treatment Products

Recombinant Factor Concentrates

Manufactured using genetically engineered cells that carry a human factor gene

During 1990s, licensed rFVIII and rFIX products became available

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Treatment Products

Recombinant Factor Concentrates con't

2nd Generation

No added human/animal proteins in final product

3rd Generation

No human and animal proteins in growth medium or final product

4th Generation

Next step — extended half-life

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FVIII New Generation & Longer Lasting Products

Manufacturer	Product	Description
Novo Nordisk	Novoeight®	B-domain truncated recombinant FVIII
Pfizer	REFACTOAF®	B-domain deleted recombinant FVIII
Octapharma	Nuwiq®	Human B-domain deleted recombinant FVIII (HEK 293 cells)
Shire (Baxalta/Baxter)	ADYNOVATE®	PEGylated Advate – recombinant FVIII
Novo Nordisk	N8-GP	GlycoPEGylated Turoctogog Alfa
Bayer	KG-N BAY 94-9027	PEGylated – domain deleted recombinant FVIII
CSL Behring	rFVIII-SingleChain CSL627	rFVIII-SingleChain (covalently bonded)
Biogen	ELOCTATE®	Recombinant FVIII FC fusion protein

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FVIII Newest Generation & Longer Lasting Products

Manufacturer	Product	Description
Genentech/Roche	HemLibra®	therapeutic antibody using the Chinese hamster ovary (CHO) cell line. Contain no human plasma nor human blood components.

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FIX New Generation & Longer Lasting Products

Manufacturer	Product	Description
Shire (Baxalta)	RIXUBIS	Recombinant FIX with reduced FIXa content
Pfizer	BeneFIX®	Recombinant FIX (CHO)
Aptevo BioTherapeutics	IXINITY®	Recombinant FIX with post translational modifications produced in genetically modified CHO cells
Novo Nordisk	N9-GP	GlycoPEGylated rFIX
Biogen	ALPROLIX®	Recombinant FIX FC fusion protein
CSL Behring	IDELVION®	Recombinant FIX Abumin fusion protein

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Alternative Treatment Products

Plasma

Cryoprecipitate

- derived from blood & contains moderately high concentration of FVIII
- effective for joint & muscle bleeds
- chance of viral contamination; challenging to store & administer
- can be made at local blood collection facilities

Fresh Frozen Plasma

- red cells removed, leaving blood proteins including FVIII and FIX
- less effective than cryoprecipitate for treating hemophilia A as FVIII is less concentrated
- large volumes of plasma must be transfused; can lead to circulatory overload
- still only product available in some countries

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Treatment Products

Treatment For Hemophilia C

Cyklokapron (Tranexamic Acid) is often used for both treatment after an incident of bleeding and as a preventive measure to avoid excessive bleeding during oral surgery.

Can use Fresh Frozen Plasma (FFP) or rec. fXI if needed.

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Factor Inhibitors

Treatment-related complication

Antibodies directed against administered factor concentrates
IgG antibodies that neutralize clotting factors
Render replacement therapy ineffective
More frequently encountered in patients with severe hemophilia
Cumulative incidence:

Hemophilia A patients

- Severe: 20 – 30%
- Moderate/mild: 5 – 10%

Hemophilia B patients

- < 5%

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Detecting Factor Inhibitors

Replacement therapy patients should be screened for inhibitor development

Confirmation of the presence of an inhibitor and quantification of the titer is performed in the laboratory, preferably using the Nijmegen-modified Bethesda assay

Srivastava, A., Brewer, A. K., Mauser-Bunschoten, E. P., Key, N. S., Kitchen, S., Llinas, A., Ludlam, C. A., Mahlangu, J. N., Mulder, K., Poon, M. C., Street, A. and Treatment Guidelines Working Group The World Federation Of Hemophilia (2013), Guidelines for the management of hemophilia. Haemophilia, 19: e1–e47. doi:10.1111/j.1365-2516.2012.02909.x

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ORIGINAL ARTICLE

Validation of Nijmegen–Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance

C. H. MILLER, S. J. PLATT, A. S. RICE, F. KELLY, J. M. SOUCIE and THE HEMOPHILIA INHIBITOR RESEARCH STUDY INVESTIGATORS*

MILLER, C. H., PLATT, S. J., RICE, A. S., KELLY, F., SOUCIE, J. M. and the Hemophilia Inhibitor Research Study Investigators* (2012), Validation of Nijmegen–Bethesda assay modifications to allow inhibitor measurement during replacement therapy and facilitate inhibitor surveillance. Journal of Thrombosis and Haemostasis, 10: 1055–1061.

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Detecting Factor Inhibitors

The Bethesda Assay

Quantitative Assays: The Bethesda Assay. The Bethesda assay is widely used to quantitate the concentration of a factor VIII inhibitor. 1 Bethesda Unit (Bu) is defined as the amount of an inhibitor that will neutralised 50% of 1 unit of FVIII:C in normal plasma after 120 minutes incubation at 37°C.

Nijmegen modified Bethesda Assay

The Nijmegen modification of the factor VIII inhibitor assay involves buffering the normal plasma with 0.1M imidazole buffer at pH7.4 and using immunodepleted factor VIII deficient plasma in the control mixture. At low inhibitor titres (<1 Bu) the classical Bethesda assay can result in false positives whereas the Nijmegen modified assays would give zero levels of inhibition.

Reference: practical-haemostasis.com/factor assays/inhibitor_assays.html

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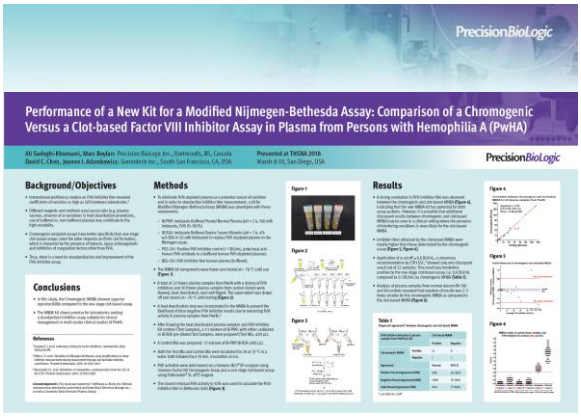
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Performance of a New Kit for a Modified Nijmegen-Bethesda Assay: Comparison of a Chromogenic Versus a Clot-based Factor VIII Inhibitor Assay in Plasma from Persons with Hemophilia A (PwHA)

PrecisionBioLogic

Background/Objectives

Methods

Results

Conclusions

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PrecisionBioLogic

Treating Factor Inhibitors

Greatest problem in the management of hemophilia today

Treatments include:

High-Dose Clotting Factor Concentrates
Bypassing Agents (e.g. NovoSeven®)
Immune Tolerance Induction (ITI) Therapy
Rituxan® (rituximab)

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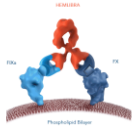
PrecisionBioLogic

Treating Factor Inhibitors

HEMLIBRA
emicizumab-kxwh
injection for subcutaneous use | 150 mg/mL

HEMLIBRA acts like a bridge, bringing activated factor IX and factor X together. This enables the clotting process to continue without needing to replace factor VIII.



HEMLIBRA does not cause or increase factor VIII inhibitors.

HEMLIBRA remains active in the presence of factor VIII inhibitors.

Since **HEMLIBRA** is not derived from blood plasma, it does not contain blood viruses.

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PrecisionBioLogic

Future Treatment Options

Spark Therapeutics

WE ARE SPARK | OUR PRODUCTS | OUR SCIENTIFIC PLATFORM & PROGRAMS | PATIENTS & FAMILIES | HEALTHCARE PROFESSIONALS

SPK-8011: Hemophilia A

Spark Therapeutics is developing *SPK-8011*, an investigational gene therapy for hemophilia A, or factor VIII deficiency. Hemophilia A is a serious and rare inherited hematologic disorder, characterized by mutations in the *F8* gene, which lead to deficient blood coagulation and an increased risk of bleeding or hemorrhaging. Spark retains global commercialization rights to its *SPK-FVIII* program includes both *SPK-8011* and *SPK-8016* for hemophilia A.

SPK-8016: Hemophilia A with inhibitors

Spark Therapeutics is developing *SPK-8016*, a novel, internally developed investigational gene therapy for hemophilia A, or Factor VIII deficiency, to address the inhibitor market using a gene-based approach. Hemophilia A is a serious and rare inherited hematologic disorder, characterized by mutations in the *F8* gene, which lead to deficient blood coagulation and an increased risk of bleeding or hemorrhaging. Inhibitors occur in as many as 30 percent of people with severe or moderately severe hemophilia A. Spark retains global commercialization rights to *SPK-8016*.

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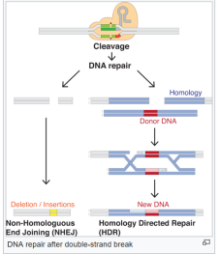
PrecisionBioLogic

Future Treatment Options

CRISPR (/ˈ krɪspər/) (**clustered regularly interspaced short palindromic repeats**)



CRISPR gene editing is a method by which the genomes of living organisms may be edited.

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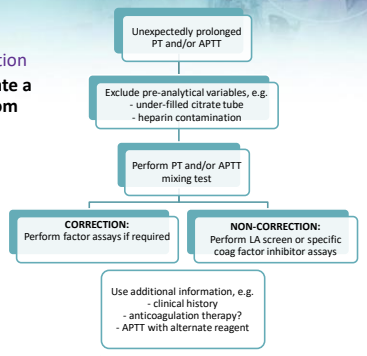
Mixing Studies

A first-line investigation

Purpose: differentiate a factor deficiency from an inhibitor



Kershaw GK, Orellana D. Mixing Tests: Diagnostic Aides in the Investigation of Prolonged Prothrombin Times and Activated Partial Thromboplastin Times. Semin Thrombos Hemost 2013;39:283–90.

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Case Study #1

8-month old male

Uncircumcised

Learning to crawl

Mother noticed swollen knee, which seemed painful and was hot to the touch

Visit with doctor revealed:

Bruising on legs and arms

No definitive family history of bleeding disorders

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8-month old male

Screening test results

Test	Patient	Normal Range
PT	12 sec	10 - 12 sec
PTT (APTT)	>120 sec	25 - 35 sec
PLT count	200,000/µL	120 - 440,000/µL
What is the next step?		

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8-month old male

Factor assay results

Factor Assay	Patient	Normal Range
FVIII	< 1%	50-150%
FIX	80%	50-150%
FXI	95%	50-150%
FXII	93%	50-150%
Factor assays show severe FVIII deficiency; referred to Hemophilia Treatment Center for treatment		

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PT and PTT Test Results in Inherited Coagulopathies

PT	PTT	Single Factor Deficiency
Long	Normal	VII
Long	Long	X, V, II and fibrinogen[1]
Normal	Long	VIII, IX, XI[2]

[1]PT & PTT prolonged when fibrinogen is <100 mg/dL, perform fibrinogen assay
[2]Contact factor deficiencies XII (1–3% prevalence), prekallikrein (PK, Fletcher), or high molecular weight kininogen (HMWK, Fitzgerald) also prolong PTT results, but no bleeding

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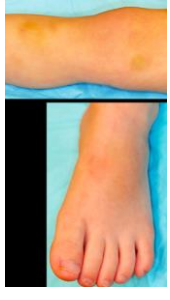
Case Study #2

2-YO male hemophilic



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Case Study #2

2-YO male hemophilic

Test	Result	Normal Range
HGB	11.8 g/dL	9.6 - 15.6 g/dL
PT	11.2 s	9.8 - 12.6 s
PTT (APTT)	65 s	25 - 35 s
PLT count	310,000/µL	150 - 400,000/µL
Fibrinogen	390 mg/dL	220 - 498 mg/dL
Inflamed, swollen knee and ankle		

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Mixing Study Result

2-YO male hemophilic

Assay	Result	Normal Range	Comment
PTT	65 s	25 - 35 s	Confirms previous PTT
PTT/control 1:1 mix immediate	33.5 s	Control 30 s	
PTT/control 1:1 mix 1 h at 37°C	47.9 s	Control 35 s	Control is incubated alone and with mix
Conclusion: Anti-FVIII inhibitor			

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Summary

Hemophilia is a rare, X-linked congenital bleeding disorder

A - FVIII deficiency

B - FIX deficiency

Bleeds can occur inside or outside the body; may begin immediately, after a delay of several hours or spontaneously

Accurate measure of factor activity is necessary to:

Make a diagnosis

Classify the severity

Monitor therapy

Factor replacement therapy is the preferred treatment

Inhibitors are now the greatest problem in the management of hemophilia

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Summary

Mixing studies are a first-line investigation into the cause of an abnormal screening test (PT or APTT)

They can be done locally to differentiate a factor deficiency from an inhibitor and guide further investigation

Patient plasma is mixed with normal plasma and screening test repeated

If results correct, suggests factor deficiency and specific factor assays can be performed

If results don't correct, suggests an inhibitor or other interference and applicable assays can be performed

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Resources

Publications

Bloody Easy: Coagulation Simplified, 2013, ORBCoN

Journal of Thrombosis and Haemostasis, Wiley

Quick Guide to Hemostasis, 2015, AACC Press

Rodak's Hematology: Clinical Principles and Applications, 2016, Elsevier

Websites

hematology.org

managedcarehemo.com

fritsmafactor.com

wfh.org

bloodcmecenter.org

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Resources

Special thank you to George Fritsma who originally authored this presentation for the Precision BioLogic webinar entitled *Improving Acute Care with Coagulation Mixing Studies*

www.fritsmafactor.com

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PrecisionBioLogic
Thank you
Jim DeMase
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Frozen
diagnostic products.
One simple
idea, many cool
benefits.

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