WEBINAR SERIES | SESSION 1

PRACTICAL EDUCATION ON BLEEDING DISORDERS

Knowledge for All

Medical educational webinar series on global topics surrounding bleeding disorders.

THURSDAY APRIL 22, 2021, 8–10 A.M. EDT

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WELCOME

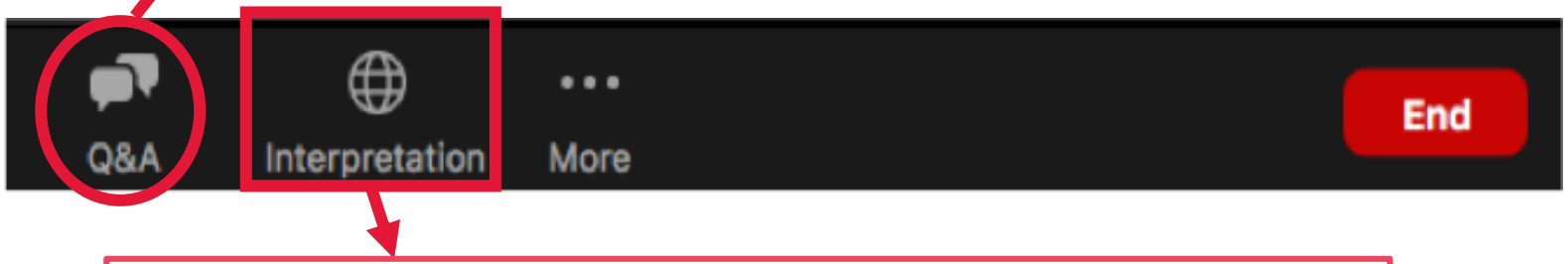
GLENN PIERCE, MD PhD

WFH VICE PRESIDENT, MEDICAL

QUESTIONS AND TRANSLATION

FOR COMPUTERS OR TABLETS

Please submit your questions in the Q&A box



Please select your language of preference now

Por favor seleccione su idioma de preferencia ahora

Veuillez sélectionner votre langue de préférence maintenant

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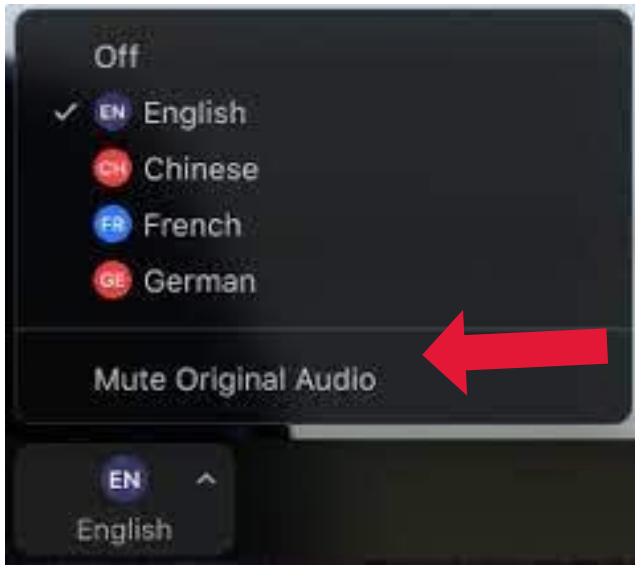
Пожалуйста выберите язык



Option to mute the original English audio

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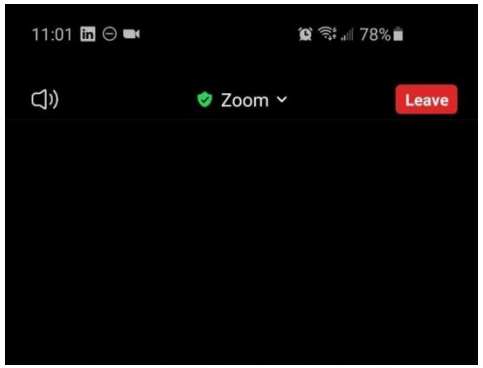
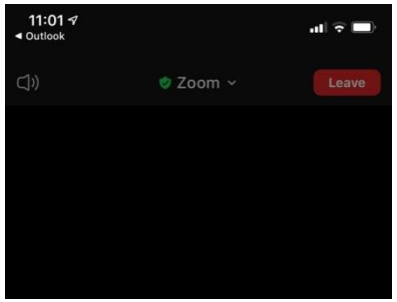
QUESTIONS AND TRANSLATION

FOR MOBILE PHONES





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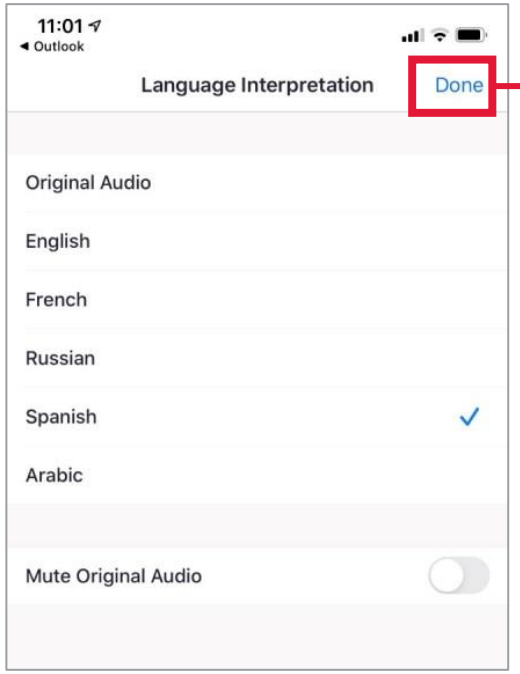

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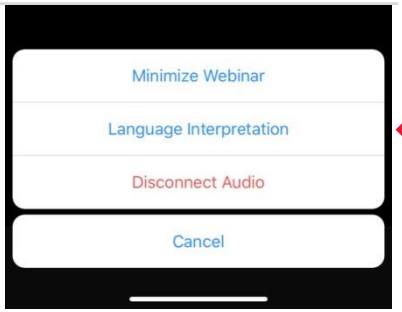

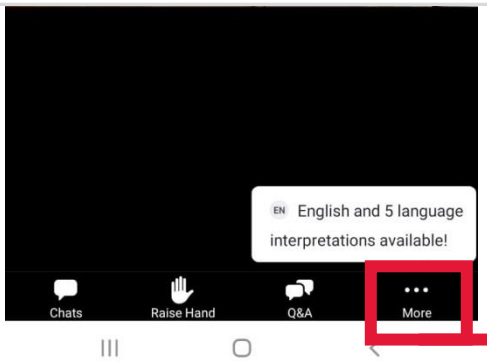

Click on the 3 dots to select the interpretation channel

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AGENDA

1. Opening & welcoming remarks
2. 20-Minute Medical Consult with a Patient: What you need to cover
3. 20-Minute MSK Consult: What you need to cover
4. Critical Lab Tests for People with Hemophilia
5. Q&A
6. Chromogenic Assays: Why are they useful in hemophilia
7. Bleeding Disorder Products: The long journey of production
8. Navigating Rebalancing Agents in Clinical Trials
9. Q&A
10. Closing remarks

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WFH Vice President, Medical
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Marlène Beijlevelt, MSc
Chair of WHF Nurses Committee
The Netherlands

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SPEAKERS

Cedric Hermans, MD, PhD
WFH Medical board member
Belgium

Gianluigi Pasta, MD
Chair of WFH MSK Committee
Italy

Sukesh Nair, MD
Professor of laboratory haematology
India

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20-Minute Medical Consult with a Patient: What you need to cover

Cedric Hermans, MD PhD

WFH Medical board member

Cliniques universitaires SAINT-LUC UCL BRUXELLES

UCLouvain

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Speaker disclosures

Shareholder	-
Grant / Research Support	Bayer, Shire, Pfizer, Novo Nordisk, Takeda
Consultant	Pfizer, Bayer, Shire, Takeda, Novo Nordisk, CSL Behring, Octapharma, Sobi Biogen, LFB, CAF-DCF, Roche
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Speaker bureau	Pfizer, Bayer, Shire, Novo Nordisk, CSL Behring, Octapharma, Sobi Biogen, LFB, Roche, CAF-DCF, Kedrion
Other	-

About your hemophilia clinic ?

- How much time per patient ?
- Do you run it jointly with the nurse, physio.. ?
- Do you adopt a holistic approach of the disease ?
- Do you use electronic patients' records ?
- Are specific modules / dashboards for hemophilia available ?
- What is your main challenge when running your clinic ?
- Do you use telemedicine ?

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Haemophilia

Haemophilia (2011), 1–7 DOI: 10.1111/j.1365-2516.2011.02671.x

REVIEW ARTICLE

Recommendations for assessment, monitoring and follow-up of patients with haemophilia

P. DE MOERLOOSE,* K. FISCHER,† T. LAMBERT,‡ J. WINDYGA,§ A. BATOROVA,¶ G. LAVIGNE-LISSALDE,** A. ROCINO,†† J. ASTERMARK‡‡ and C. HERMANS§§

**University Hospital and Faculty of Medicine, Geneva, Switzerland; †Van Creveldkliniek, and Julius Center for Health Sciences and Primary Care, University Medical Center, Utrecht, Netherlands; ‡CHU Kremlin – Bicètre, Paris, France; §Institute of Haematology and Transfusion Medicine, Warsaw, Poland; ¶Department of Hematology and Transfusion Medicine, National Haemophilia Center, University Hospital, Bratislava, Slovakia; **Centre de Traitement des Hémophiles, Hôpital de Montpellier, France; ††Haemophilia and Thrombosis Center, San Giovanni Bosco Hospital, Naples, Italy; ‡‡Centre for Thrombosis and Hemostasis, Skåne University Hospital Malmö, Sweden; and §§Haemostasis-Thrombosis Unit, Division of Haematology, Cliniques Universitaires Saint-Luc, Brussels, Belgium*

Domains that are important to cover during the clinic

1. Baseline information
2. Baseline up-date
3. Treatment
4. Inhibitor
5. Bleeding
6. Joint status and pain
7. Co-morbidities
8. Dental Care
9. Physical Activities
10. Social Participation
11. Quality of Life
12. Empowerment

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Baseline Information
(key for registries)

- Date of Birth
- Blood Group
- Diagnosis
  - Hemophilia type and severity
  - Age at diagnosis
  - Family history of hemophilia
  - Age and location of first bleed (specify type of bleed)
  - Age of first joint bleed
  - Age at first substitution (specify type of substitution)
  - Genetic variant
  - Inhibitor status
- Ethnicity
- Age at start of prophylaxis
- Family history of inhibitors

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Baseline up-date
(at each clinic)

- Date of update
- Replacement therapy
  - Recovery and half life of FVIII/FIX
- DDAVP
  - Response
- Family history (Family Tree)
  - New cases
  - New inhibitors
  - Carriers
- Infections
  - Blood borne
  - Other
- Vaccinations
- Major Events since last visits
  - Major bleeding
  - Other
- Cumulative exposure to FVIII-FIX (EDs)
- Inhibitor screening
  - At least every 5 EDs until 50 EDs
  - At least 2x year until 150 EDs
  - Annually thereafter

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Hemostatic treatment information

<table>
<tr><td colspan="2">Therapy prescribed
Concentrate / Non-factor / other,</td></tr>
<tr><td>Treatment regimen : On-demand
• Dosing
• Frequency
Annual consumption</td><td>Treatment regimen : Prophylaxis
• Dosing
• Frequency
Annual consumption</td></tr>
<tr><td colspan="2">Examine Log-Book:
• Dose and time of each infusion / reason / adherence
• Batch number
Other hemostatic treatments (DDAVP, antifibrinolytic agents,…)
Hospitalisations (reason)
Other medications
Understanding of treatment modalities, awareness of treatment innovations / treatment alternatives / Motivation and eligibility for trials</td></tr>
</table>

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Information on bleeding episodes

- Bleeding pattern is the key parameter to evaluate efficacy of treatment strategy
- Any changes in bleeding frequency or severity should be thoroughly investigated and treatment modified as necessary

The ambition is to have ZERO spontaneous joint bleed

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Information on bleeding episodes

- **Major hemarthrosis**
  - Number of episodes
  - Time, location and reason
- **Minor hemarthrosis**
  - Number
  - Time, location and reason
- **Other major bleeds**
  - Number
  - Time, location and reason

Assessment of joint health status and pain

- The treatment should aim at preserving the joints
- Objective assessment of joint function and pain is central to maintain joint health
- Assessment should identify target joint(s), problem joint(s), early joint damage
- Painful symptoms should be recorded

Joint assessement requires the involvement of a dedicated physiotherapist with expertise in hemophilia

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Screening of co-morbidities (mainly in adult patients)

- Liver disease (HCV) – Long-term follow-up required even after eradication
- HIV
- Cardio-vascular risk factors or diseases ?
- Cancer (prostate ?, other ?)
- Other health problems
- Osteoporosis
- Sexual dysfunction
- Obesity
- Liver steatosis
- ….

Comorbidities should be identified

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Dental care

- Last dental assessment ?
- Dental hygiene ?
- Adequate coverage is required prior to, and possibly after, more intensive treatments (deep cleaning, removal of heavy plaque / tartar)

Physical activities

- Hemophilia Activities List (HAL) – *Hemophilia specific self assessment questionnaire to assess a patient's self-perceived functional ability*
- 1x year
- Sports activities / Hobbies
  - Which sports
  - Frequency
  - Any regimen of concentrate use before sports
- Occupation
  - Risk factors for bleeding or joint damage
  - Limitations of current condition on type of work undertaken

Evaluation of quality of Life

- QOL measures can help patients and clinicians to decide between different treatments, to monitor treatment success from the patients' perspective and increasingly they are being used to support funding and re-imbursement for drugs

- The EQ-5D is simple, short and allows comparisons between different medical conditions.
  - However, the EQ-5D is a very general instrument and has been criticised on the basis that it was developed in healthy people, asking them to imagine a poor health state.
  - So for some circumstances a more hemophilia and age specific tool may be desirable

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Social participation

- Education
- Employment status
- Marital Status
- Offspring
- Disability Allowance
- Days lost from work or school since last visit
- Review the total days lost annually to assess overall impact on patient
- Involvement in a patient organisation / Interactions with NMO, WFH, EHC,...

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Patients’ empowerment

- Understanding of diseases, treatment, innovations
- Acceptance of the disease ?
- Interactions with NMO,
- Knowledge about WFH, EHC activities / initiatives
- Interest for clinical trials
- Inclusion in registry (WBDR)
- Interactions with other patients

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How to improve your clinic: some personal tips ?

- Review patients' medical records before the clinic
- Short list the priorities / active problems that will require attention
- Contact the patient before the clinic to identify new active problems
- Holistic approach of hemophilia
- Value each clinic as opportunities to improve understanding of the disease, adherence, interaction with patients' associations…(patients' empowerment)
- Run your clinics with the nurse and the physiotherapist
- Avoid patients' monopoly – share patients with colleagues.

THANK YOU

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20-Minute MSK Consult: What you need to cover

Gianluigi Pasta, MD
Chair of WFH MSK Committee
Orthopedic and Traumatology Department
Fondazione IRCCS Policlinico San Matteo Pavia, Italy

Fondazione IRCCS Policlinico San Matteo

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HA and MSK expert

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HA and MSK expert

HA and MSK expert

- Pathogenesis
- Diagnosis
- Treatment

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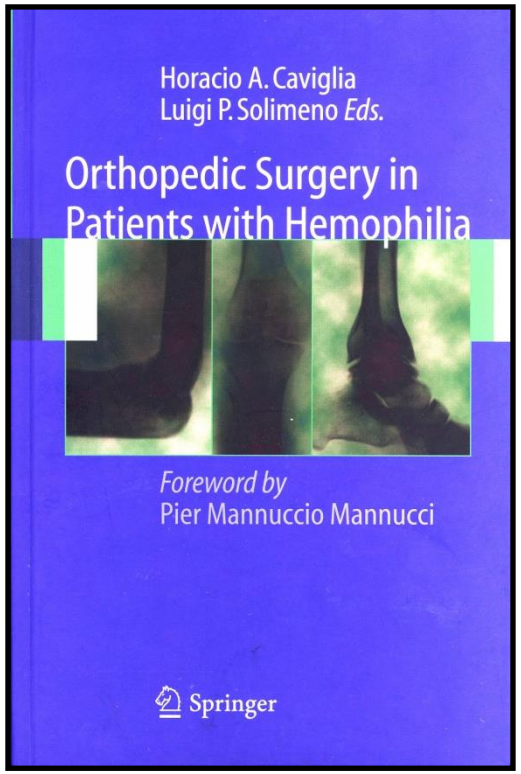
Musculoskeletal assessment



Listen to the patient and perform and adequate physical exam.

Gilbert M, Solimeno L, Caviglia H.

Musculoskeletal assessment

“ Assessment of impairment and function is essential in order to monitor joint status and evaluate therapeutic interventions in patients with haemophilia.”

Beeton K et al., Haemophilia 2006

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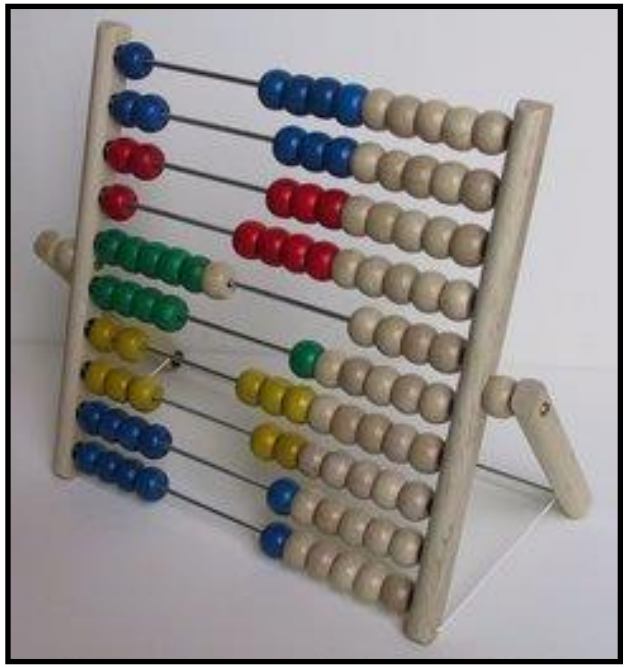
Musculoskeletal assessment



Arthritis Care & Research
Vol. 63, No. 2, February 2011, pp 223–230
DOI 10.1002/acr.20353

ORIGINAL ARTICLE

Validation of a New Pediatric Joint Scoring System From the International Hemophilia Prophylaxis Study Group: Validity of the Hemophilia Joint Health Score

BRIAN M. FELDMAN,[1] SHARON M. FUNK,[2] BRITT-MARIE BERGSTROM,[3] NICHAN ZOURIKIAN,[4] PAMELA HILLIARD,[1] JANJAAP VAN DER NET,[5] RAOUL ENGELBERT,[6] PIA PETRINI,[3] H. MARIJKE VAN DEN BERG,[7] MARILYN J. MANCO-JOHNSON,[2] GEORGES E. RIVARD,[8] AUDREY ABAD,[9] AND VICTOR S. BLANCHETTE[1]

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Musculoskeletal assessment

Subject ID # :_____ Name of Physiotherapist:_____

Assessment # : _____ Date: _____
yyyy / mm / dd

Time: _____

Hemophilia Joint Health Score 2.0

	LE	RE	LK	RK	LA	RA
Duration (swelling)						
Swelling						
Muscle Atrophy						
Crepitus on motion						
Flexion Loss						
Extension Loss						
Joint Pain						
Strength						
Joint Total						

Global Gait Score ☐

Total Score (Sum of joint totals + global gait score) ☐

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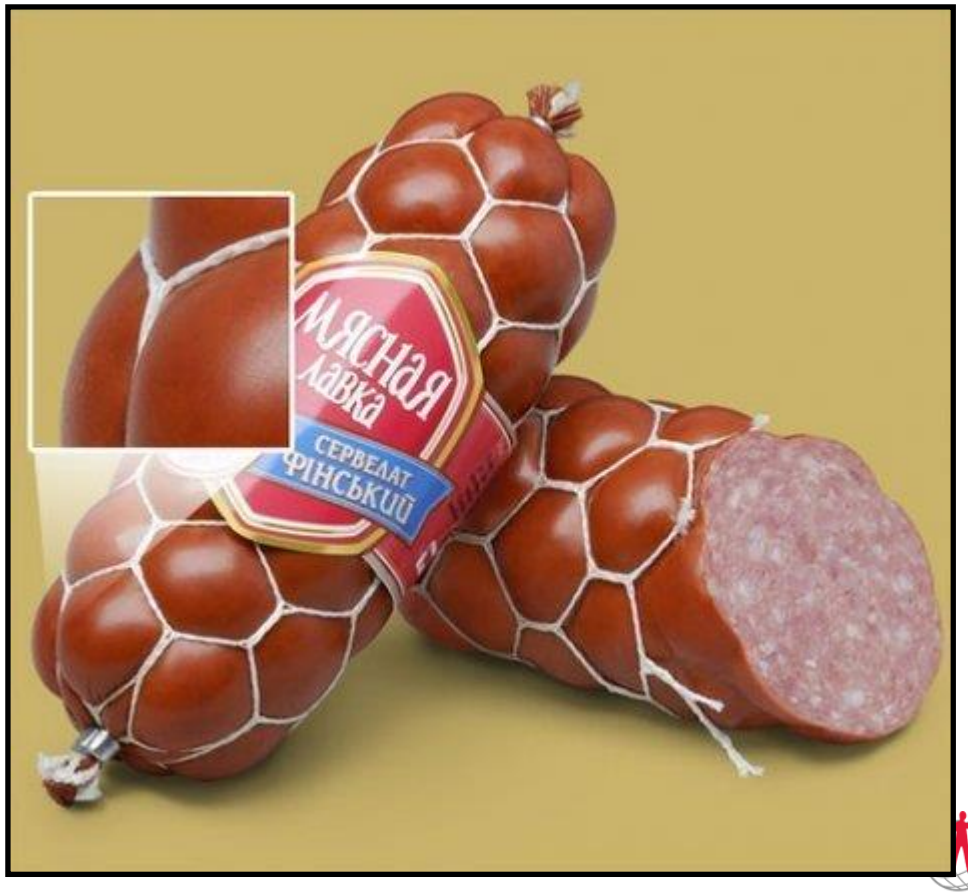
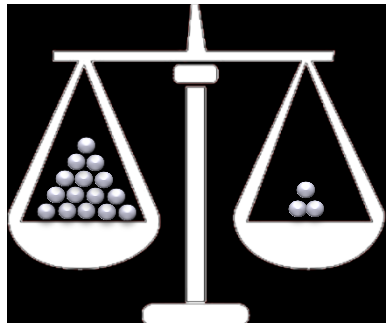
Musculoskeletal assessment



Ultrasound

Ultrasound



PROS

- Excellent spatial resolution
- Dynamic capabilities *(joint motion, muscle contraction)*
- Clinical examination *(US performed by physicians!)*
- Availability, portability, low cost

CONS

- Limited FOV *focused examination*
- Unable to examine relevant structures *(e.g. bone …)*
- Highly operator dependent *long learning curve*
- Difficult to be interpreted by the referring physician
- Reimbursement issues

Ultrasound

Development and definition of a simplified scanning procedure and scoring method for Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US)

Carlo Martinoli[1]; Ornella Della Casa Alberighi[3]; Giovanni Di Minno[5]; Ermelinda Graziano[6]; Angelo Claudio Molinari[4]; Gianluigi Pasta[7]; Giuseppe Russo[1]; Elena Santagostino[8]; Annarita Tagliaferri[9]; Alberto Tagliafico[2]; Massimo Morfini[10]

[1]Radiologia – DISSAL, Università di Genova, Italy; [2]Istituto di Anatomia Umana – DIMES, Università di Genova, Italy; [3]Direzione Scientifica – Unità di Farmacologia Clinica e Sperimentazioni Cliniche, IRCCS Istituto „G. Gaslini", Genova, Italy; [4]Unità di Trombosi ed Emostasi – IRCCS Istituto „G. Gaslini", Genova, Italy; [5]Dipartimento di Medicina Clinica e Sperimentale. Università di Napoli, Italy; [6]Dipartimento di Medicina – Scienze dell'Invecchiamento. Università di Chieti-Pescara, Italy; [7]Dipartimento di Ortopedia, Policlinico Universitario di Milano, Italy; [8]Centro di Emofila e Trombosi „AB Bonomi", Policlinico Universitario di Milano, Italy; [9]Dipartimento di Medicina Interna – AOU Parma, Italy; [10]Centro Emofilia – AOU "Careggi" Firenze, Italy

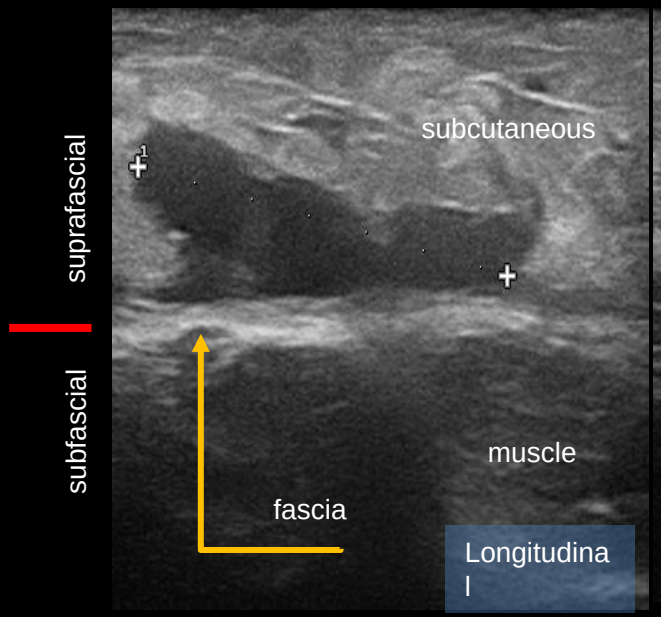
Ultrasound

- Joint bleed
- Joint effusion
- Synovitis
- Cartilage damage

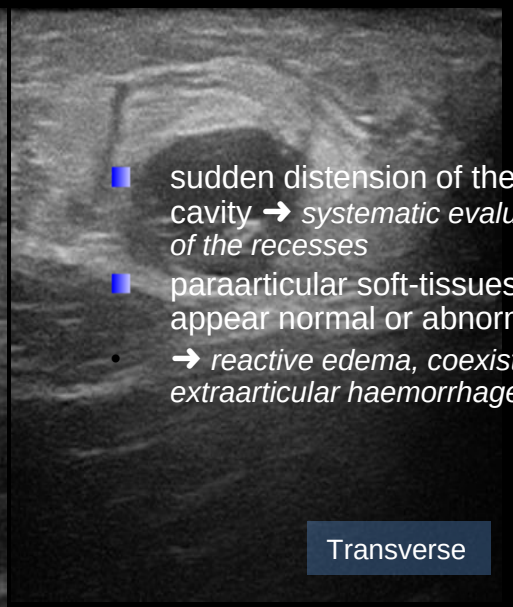
Bleed location

extraarticular

intraarticular

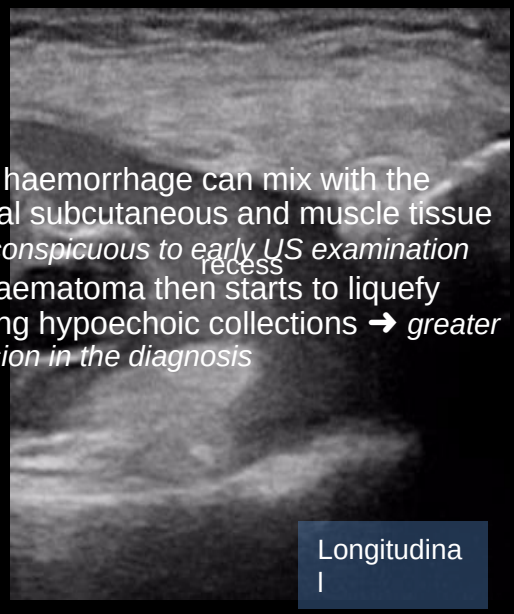


- sudden distension of the joint cavity ➜ *systematic evaluation of the recesses*
- paraarticular soft-tissues may appear normal or abnormal ➜ *reactive edema, coexisting extraarticular haemorrhage*



fresh haemorrhage can mix with the normal subcutaneous and muscle tissue ➜ *inconspicuous to early US examination*

the haematoma then starts to liquefy forming hypoechoic collections ➜ *greater precision in the diagnosis*



Joint Bleed *vs.* Osteoarthritis

Haemophilia
The Official Journal of the World Federation of Hemophilia, European Association for Haemophilia and Allied Disorders and the Hemostasis & Thrombosis Research Society
Haemophilia (2013), 1–9 DOI: 10.1111/hae.12175

ORIGINAL ARTICLE

Rapid musculoskeletal ultrasound for painful episodes in adult haemophilia patients

A. CEPONIS,* I. WONG-SEFIDAN,† C. S. GLASS† and A. VON DRYGALSKI†‡
*Dept of Medicine, Div of Rheumatology, University California San Diego, San Diego, CA, USA; †Dept of Medicine, Div of Hematology/Oncology, University California San Diego, San Diego, CA, USA; and ‡Dept of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA

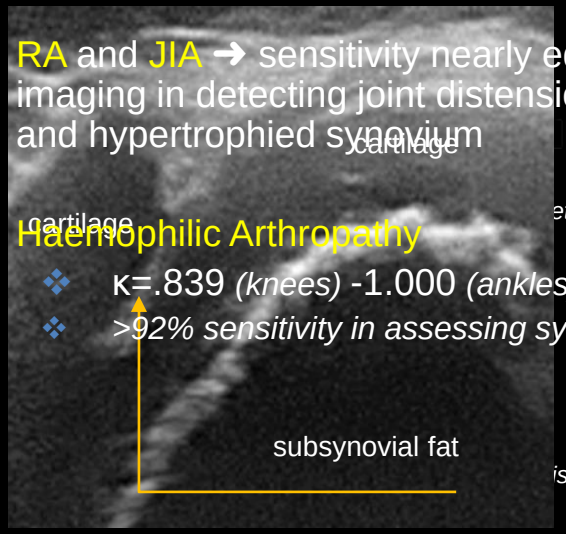
PAINFUL EPISODES

bleeding	33 →	12
arthritis-mediated	5	
undecided	2	

Ultrasound detection threshold for joint effusion

- Knee ➜ *4-7ml*
- Ankle ➜ *2ml* — *Hong et al., Am J Phys Med Rehab 2003*; *Delaunoy et al. 2003*
- Elbow ➜ *1-3ml* — *Jacobson et al., AJR 1998*

De Maeseneer et al., Invest Radiol 1998



RA and JIA ➜ sensitivity nearly equal to MR imaging in detecting joint distension by effusion and hypertrophied synovium

et al. Ann Rheum Dis 2005

Haemophilic Arthropathy

- κ=.839 *(knees)* -1.000 *(ankles)*
- *>92% sensitivity in assessing synovial hypertrophy*

isa et al. Haemophilia 2014
Doria et al. AJR, 2015

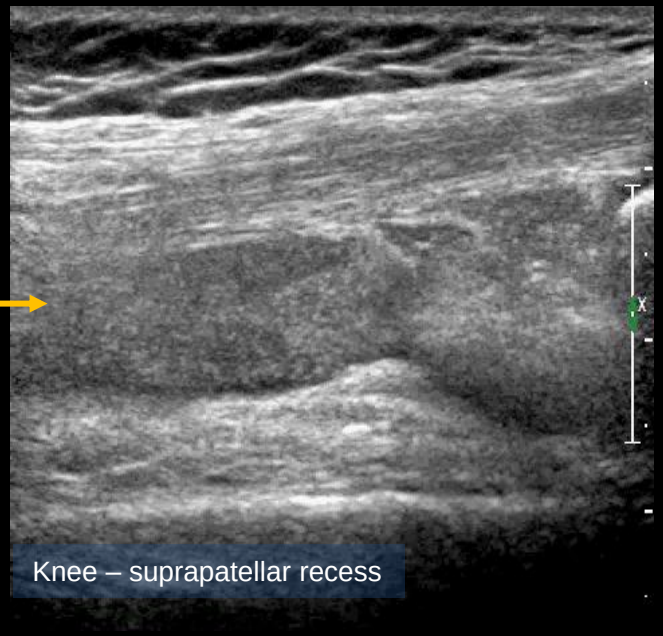
Intraarticular blood

How to distinguish blood clots from synovium?

Early stages of a bleeding episode (within 24 hours)

- homogeneous echogenic pattern *(piles of RBCs)*
- probe compression ➜ swirling motion, squeezes blood away

suprapatellar recess

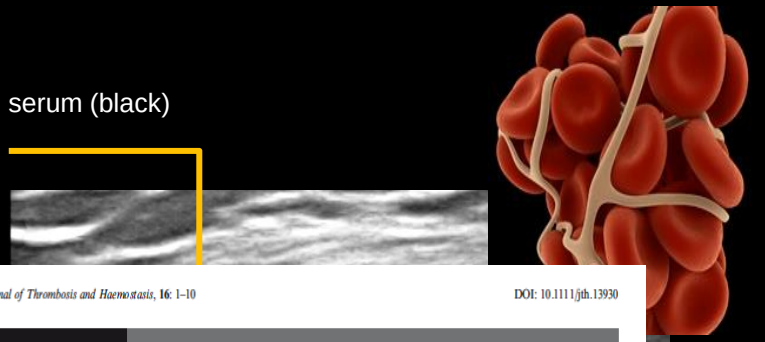


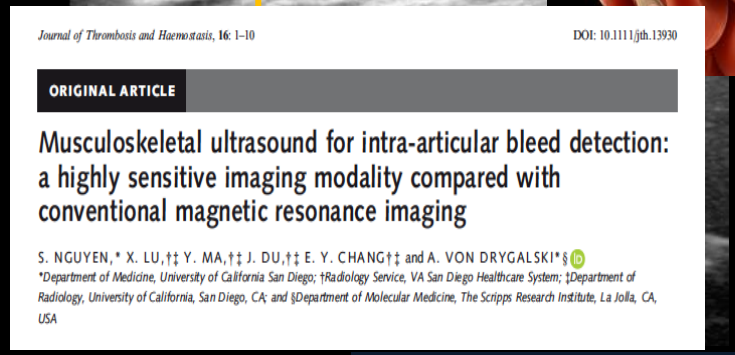
Knee – suprapatellar recess

Later stages ➜ clot formation

- fresh clots are echogenic
- clots become hypoechoic with progress
  lysis of the RBCs

serum (black)





Journal of Thrombosis and Haemostasis, **16**: 1–10 DOI: 10.1111/jth.13930

ORIGINAL ARTICLE

Musculoskeletal ultrasound for intra-articular bleed detection: a highly sensitive imaging modality compared with conventional magnetic resonance imaging

S. NGUYEN,* X. LU,†‡ Y. MA,†‡ J. DU,†‡ E. Y. CHANG†‡ and A. VON DRYGALSKI*§
*Department of Medicine, University of California San Diego; †Radiology Service, VA San Diego Healthcare System; ‡Department of Radiology, University of California, San Diego, CA; and §Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA, USA

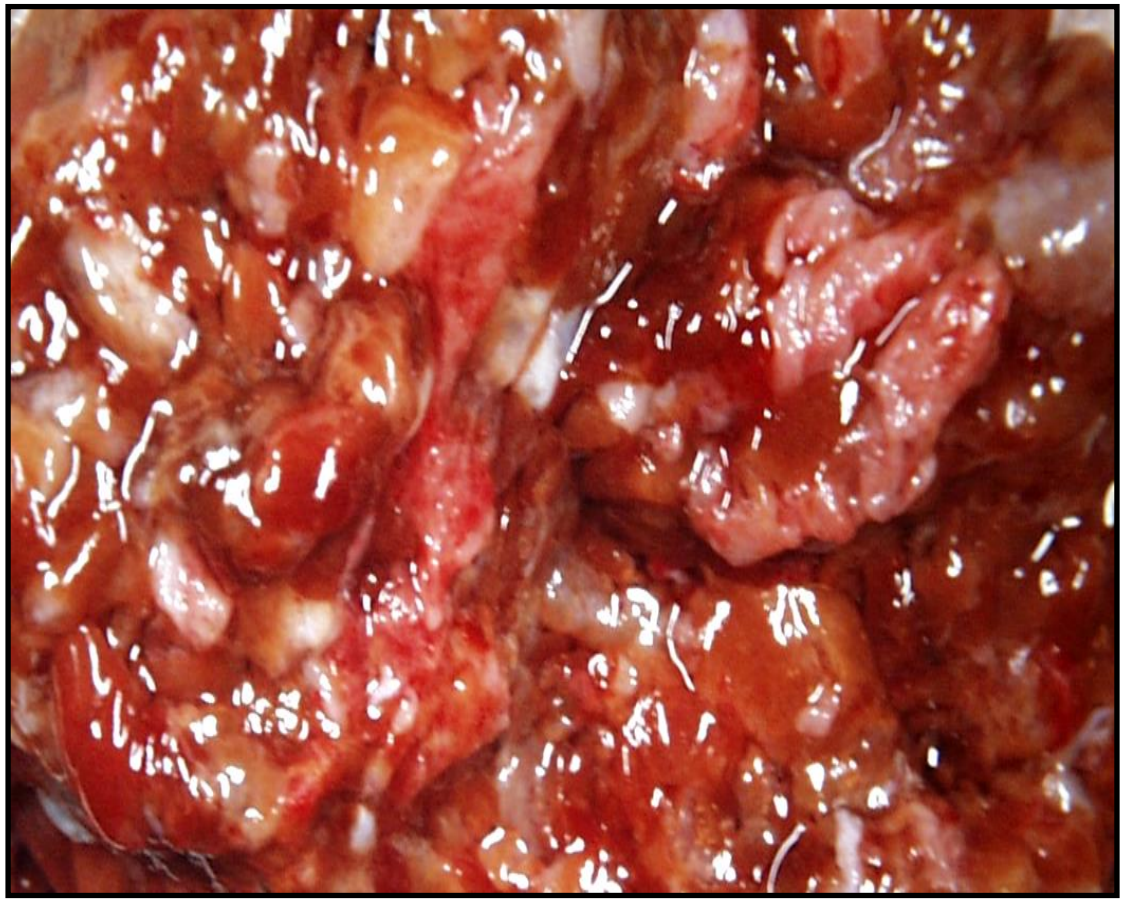
Knee – suprapatellar recess

Nguyen et al., J Thromb Haemostas 2018

Ultrasound

- Joint bleed
- Joint effusion
- Synovitis
- Cartilage damage

Diagnosis of synovitis



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Musculoskeletal assessment

Subject ID # :_____ Name of Physiotherapist:_____

Assessment # : _____ Date: _____
yyyy / mm / dd

Time: _____

Hemophilia Joint Health Score 2.0

	LE	RE	LK	RK	LA	RA
Duration (swelling)						
Swelling						
Muscle Atrophy						
Crepitus on motion						
Flexion Loss						
Extension Loss						
Joint Pain						
Strength						
Joint Total						

Global Gait Score ☐

Total Score (Sum of joint totals + global gait score) ☐

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Synovial proliferation

Haemophilia
The Official Journal of the World Federation of Hemophilia,
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LETTERS TO THE EDITORS e141

Comparing findings of routine Haemophilia Joint Health Score and Haemophlia Early Arthropathy Detection with UltraSound assessments in adults with haemophilia

M. A. TIMMER,* W. FOPPEN,† R. E. G. SCHUTGENS,* M. F. PISTERS‡§ and K. FISCHER*
*Van Creveldkliniek; †Department of Radiology; ‡Physical Therapy Research, Department of Rehabilitation, Physical Therapy Science and Sport, Brain Center Rudolf Magnus University Medical Center Utrecht; and §Center for Physical Therapy Research and Innovation in Primary Care, Leidsche Rijn Julius Health Care Centers, Utrecht, The Netherlands

Timmer et al., Haemophilia 2017

- Strong correlation between HJHS 2.1 and HEAD-US for all joints (r=0.88)
- Discrepancies between HJHS 2.1 and HEAD-US found in 7% of cases
- 14/76 joints *(n=6 elbows, n=2 knees, n=6 ankles)* showed synovial hypertrophy on HEAD-US without signs of swelling on HJHS

Haemophilia
The Official Journal of the World Federation of Hemophilia,
European Association for Haemophilia and Allied Disorders and the Hemostasis & Thrombosis Research Society

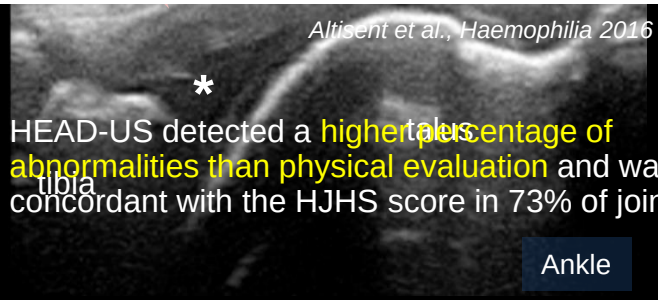
Haemophilia (2016), 22, 218–224 DOI: 10.1111/hae.12792

ORIGINAL ARTICLE *Clinical haemophilia*

Early prophylaxis in children with severe haemophilia A: clinical and ultrasound imaging outcomes

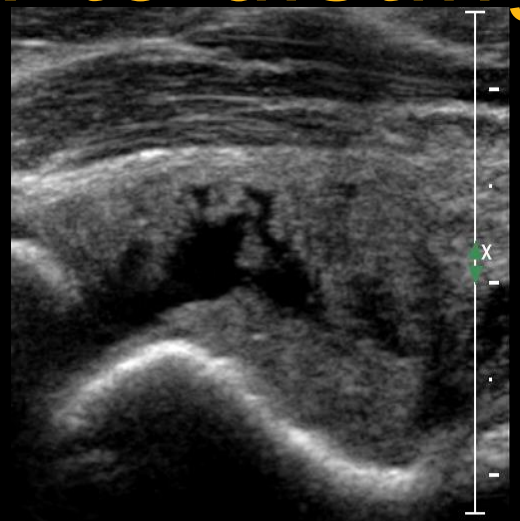
C. ALTISENT,* M. MARTORELL,* A. CRESPO,† L. CASAS,‡ C. TORRENTS‡ and R. PARRA*
*Haemophilia Centre; †Department of Rehabilitation; and ‡Department of Radiology, Hospital Universitari Vall d'Hebron, Barcelona, Spain

Altisent et al., Haemophilia 2016



- HEAD-US detected a higher percentage of abnormalities than physical evaluation and was concordant with the HJHS score in 73% of joints

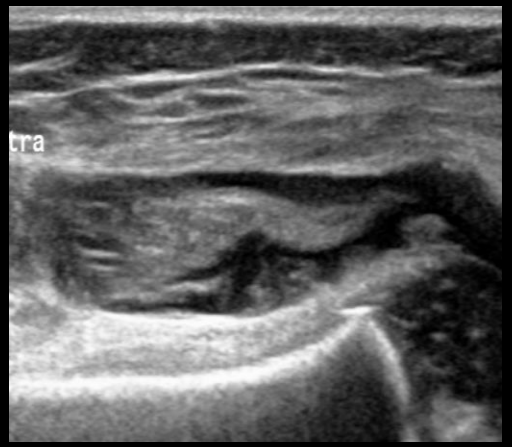
How to distinguish blood from synovium



Main Differential Features

SYNOVIAL PROLIFERATION

- attached to the walls (peripheral)
- irregular margins
- some vasculature may be detected

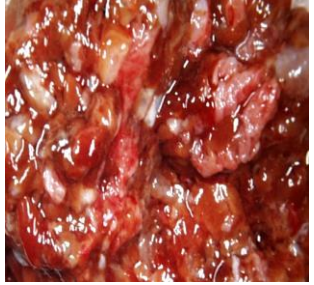


BLOOD CLOTS

- detached from the walls (central)
- smooth margins
- free of color flow signals at Doppler imaging

- In joints with preexisting synovitis such differentiation may not be straightforward

JOINT VOLUME CHANGE



Treatment of synovitis

Grade I	Grade II	Grade III	Grade IV
Transitory synovitis Back to normal between bleeds	Chronic synovitis Does not return to normal between bleeds	Chronic arthropathy, axial deformities, rigidity	Bony or fibrous ankylosis
>3bleeds in 6m Intervention	Intervention	Caution! Unlikely to succeed.	Not indicated.

Fernández-Palazzi F. Haemophilia, 1998

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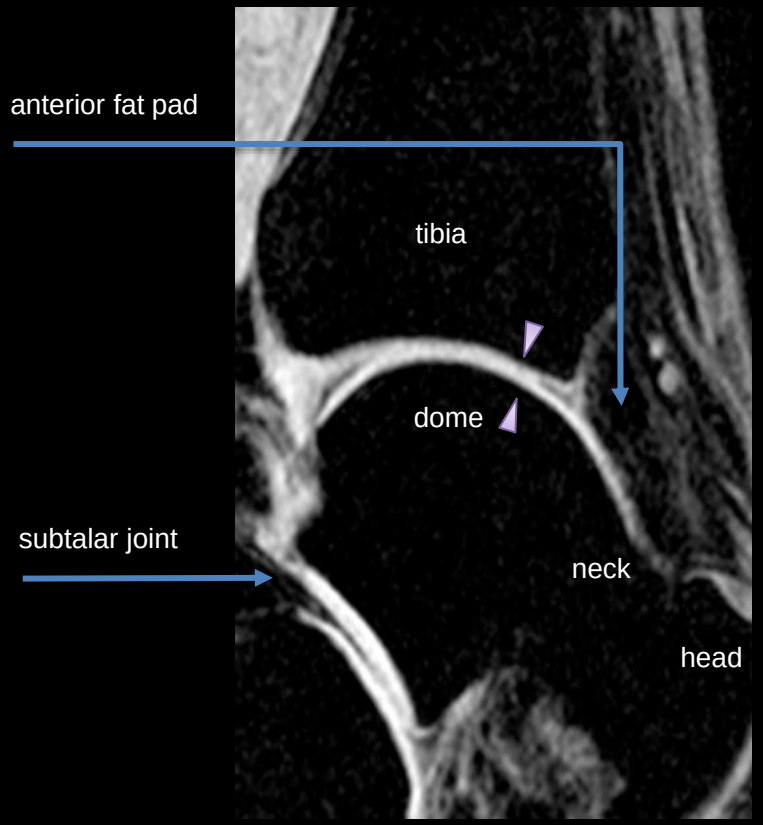
Ultrasound

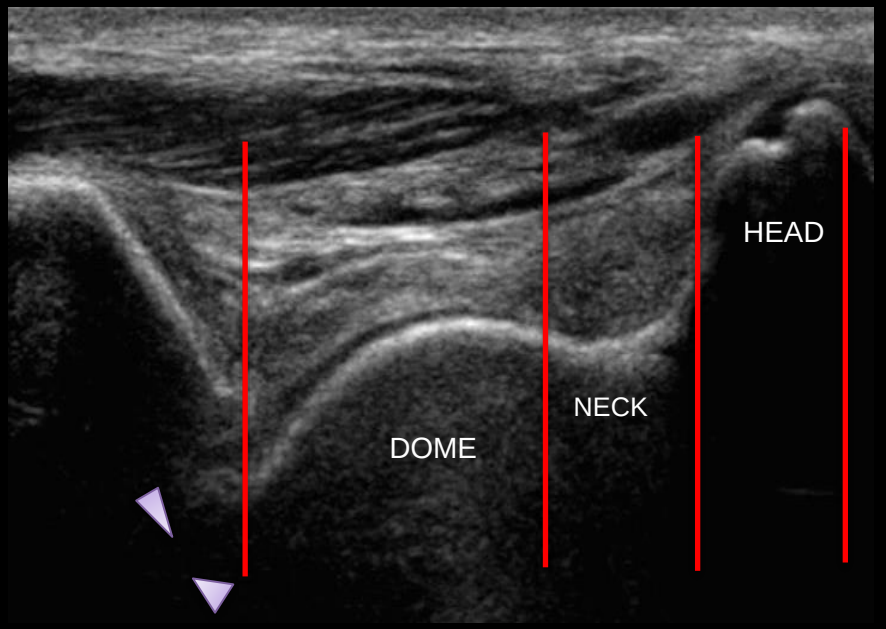
- Joint bleed
- Joint effusion
- Synovitis
- Cartilage damage

Osteochondral damage

MR imaging

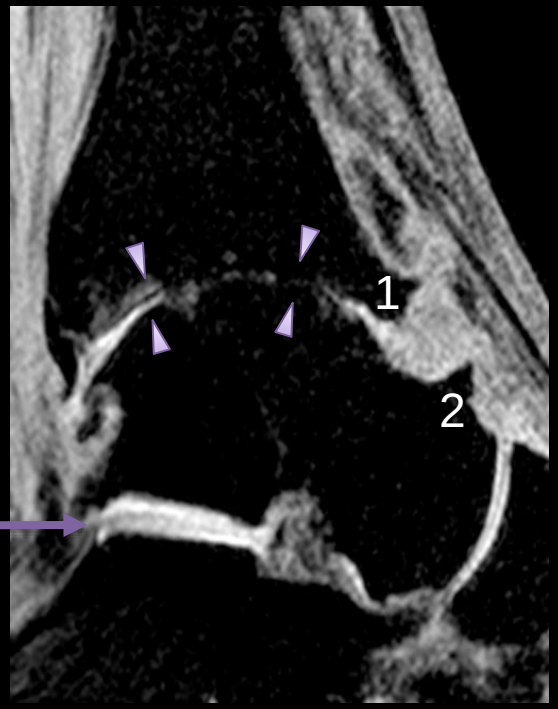


ULTRASOUND

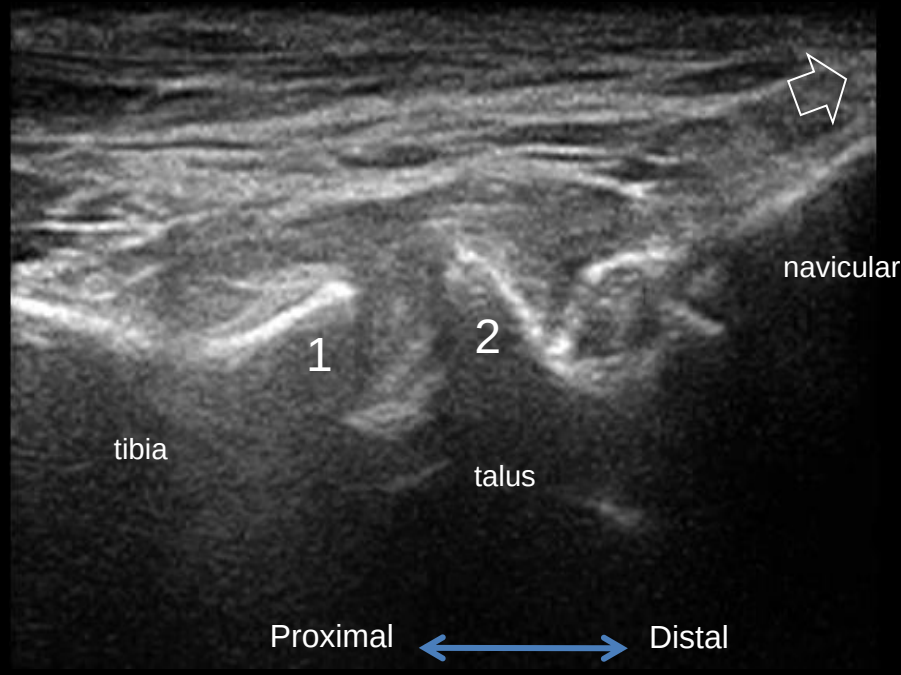


Osteochondral damage

MR imaging



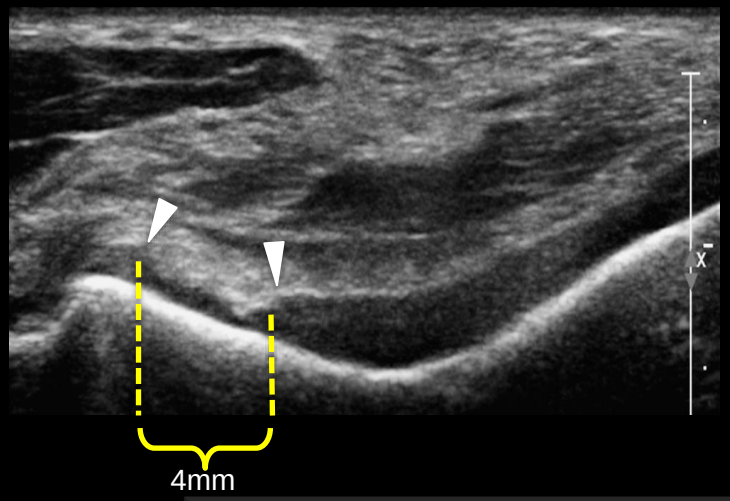
ULTRASOUND



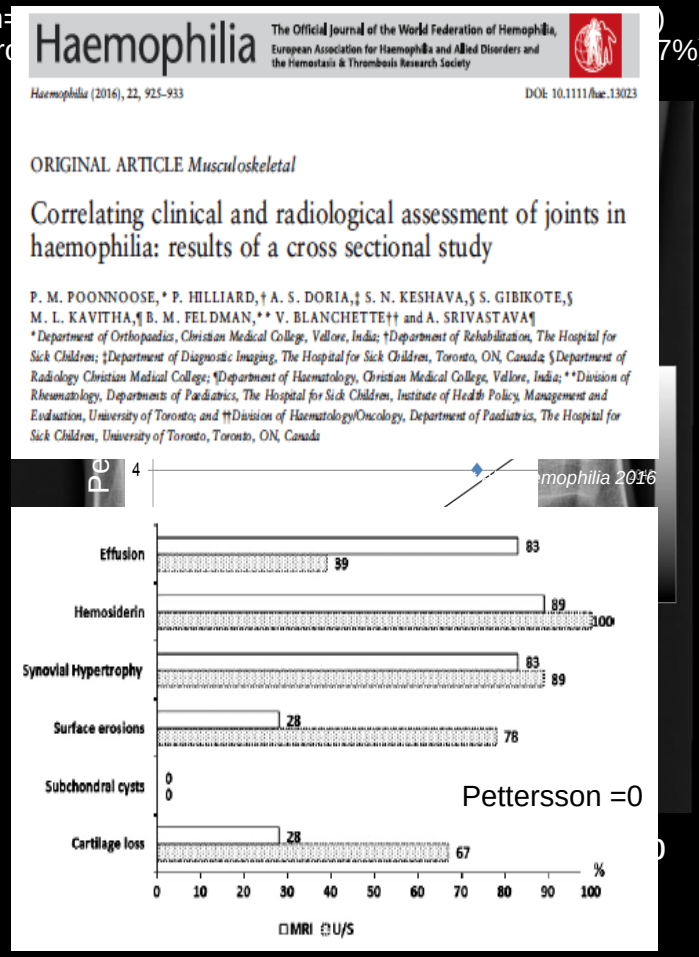
Osteochondral damage

- Compared to radiography, HEAD-US has demonstrated higher sensitivity to detect early damage signs
- Good correlation between ultrasound and MR imaging in the evaluation of bone and cartilage abnormalities in the elbows, knees and ankles

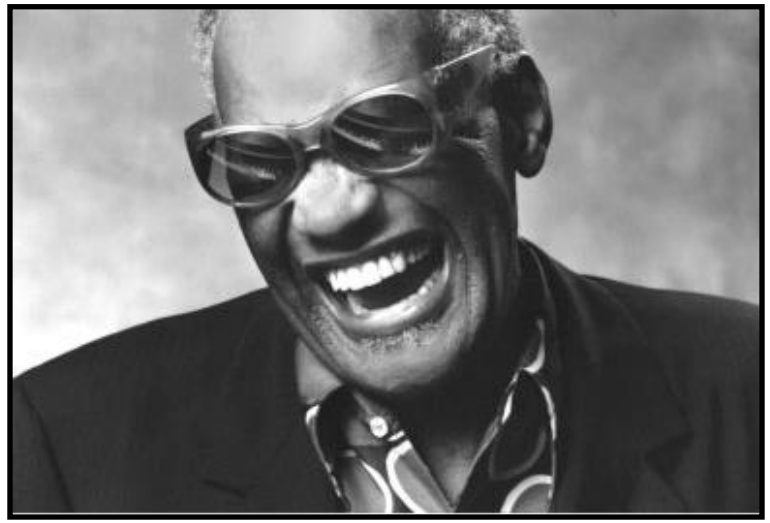
Di Minno et al. Haemophilia 2013
Sierra Aisa et al. Haemophilia 2014



- n= fr 7%)



Musculoskeletal assessment



Small changes in function can produce enormous joint loading that is not detectable by clinical and radiological examination.

Seuser A et al., 2000

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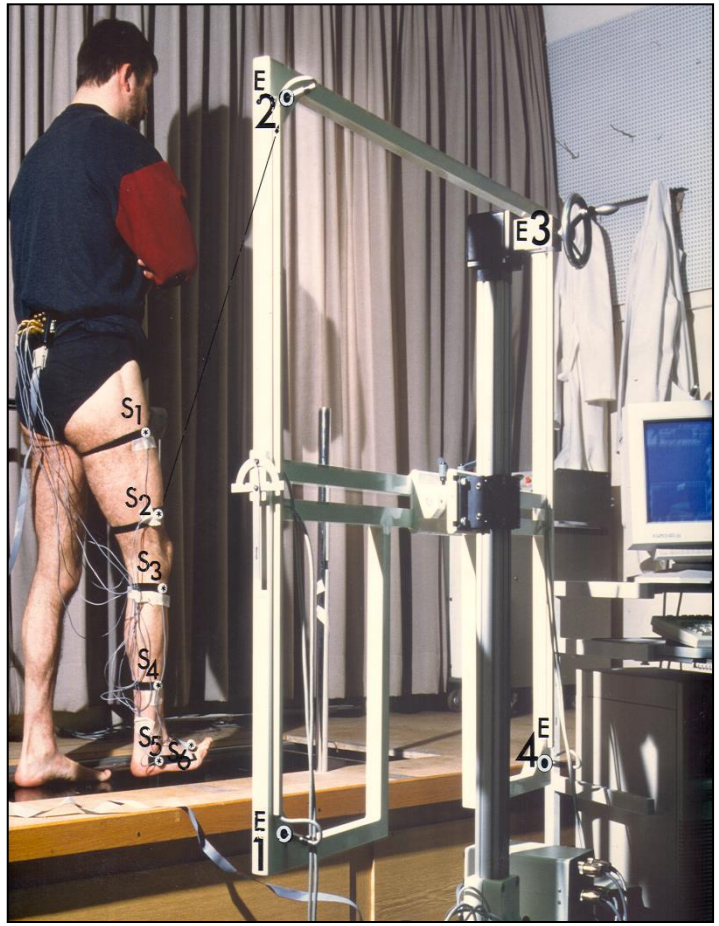
Musculoskeletal assessment



Long-term kinematic monitoring has shown that individual treatment programs can help to improve internal kinematics and thus help to preclude joint interventions later on.

Seuser A., 2008

Musculoskeletal assessment

- Costs
- Time
- Space

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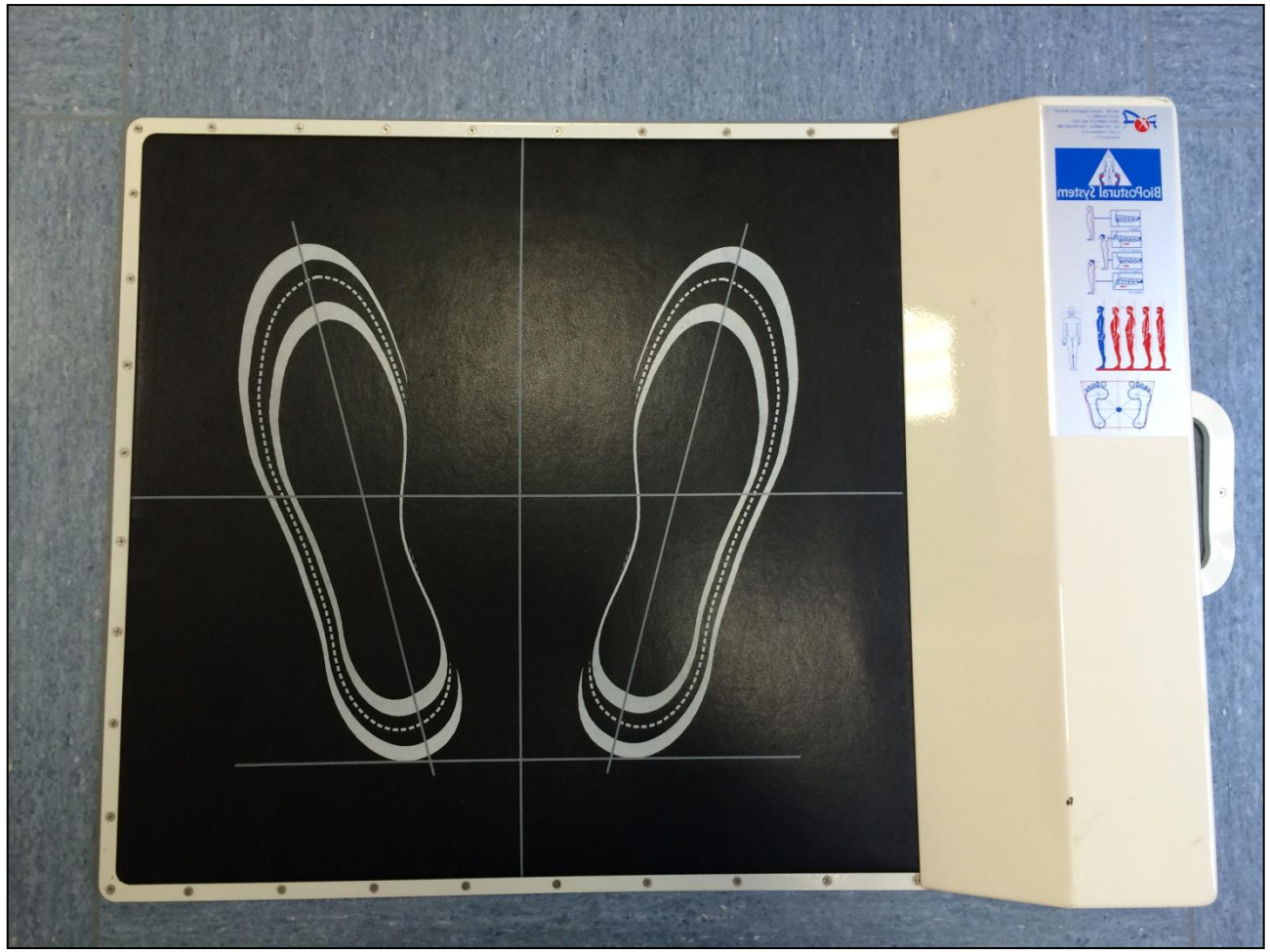
Musculoskeletal assessment



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Musculoskeletal assessment

Haemophilia
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the Haemostasis & Thrombosis Research Society

Haemophilia (2014), 20, 263–267 DOI: 10.1111/hae.12369

ORIGINAL ARTICLE *Musculoskeletal*

Integrated postural analysis in children with haemophilia

E. BOCCALANDRO,* G. PASTA,† P. M. MANNUCCI,‡ E. SANTAGOSTINO,* F. PEYVANDI,* A. SEUSER,§ M. E. MANCUSO* and L. P. SOLIMENO†

**Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre; †Department of Orthopaedic Surgery and Traumatology; ‡Scientific Direction, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy; and §Private Practice for Prevention, Rehabilitation and Orthopaedics, Bonn, Germany*

Conclusions

- Diagnosis
- Treatment

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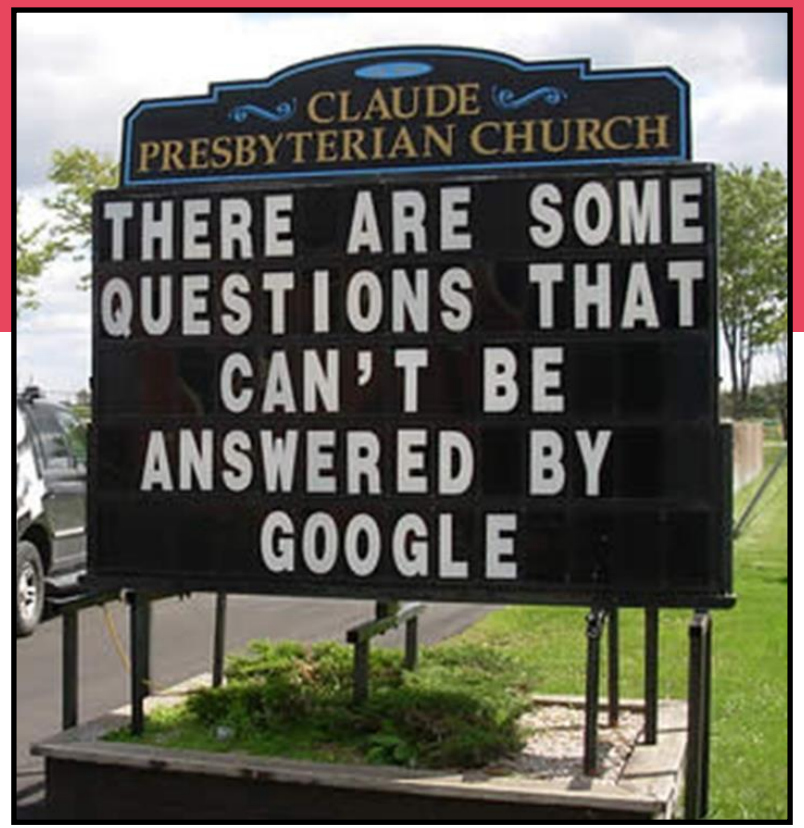
THANK YOU



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OVERCOMING CHALLENGES IN MANAGING LABORATORY DIAGNOSIS FOR BLEEDING DISORDERS

EMPHASIS ON HEMOPHILIA

Sukesh C Nair MD FRACP

Christian Medical College

Vellore, India

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DISCLOSURES FOR SUKESH C NAIR

Research Support/P.I.	No relevant conflicts of interest to declare
Employee	No relevant conflicts of interest to declare
Consultant	No relevant conflicts of interest to declare
Major Stockholder	No relevant conflicts of interest to declare
Speakers Bureau	No relevant conflicts of interest to declare
Honoraria	No relevant conflicts of interest to declare
Scientific Advisory Board	No relevant conflicts of interest to declare

Presentation includes discussion of the following off-label use of a drug or medical device: <N/A>

COAGULATION CASCADE: THE “TEXTBOOK VERSION”

INTRINSIC PATHWAY

CONTACT

XII → XIIA

PK

HMWK

XI → (HMWK) → XIA

XIA → IX

EXTRINSIC PATHWAY

TISSUE FACTOR

VII → VIIA

IX + VIIA → IXA + Ca + VIII:C + PHOSPHOLIPID *

X → XA + Ca + V + PHOSPHOLIPID *

II → IIA

I → IA

IA → FIBRIN POLYMER

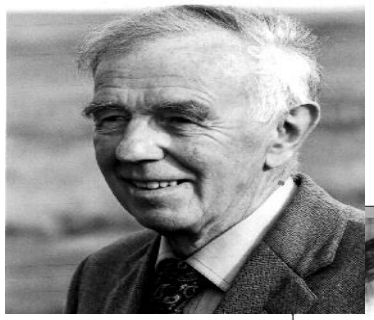

Figure 1: Dr. Oscar Ratnoff drawing blood from Mr. John Hageman. Courtesy of Dr. Ratnoff.

* Phospholipid from platelet substitute

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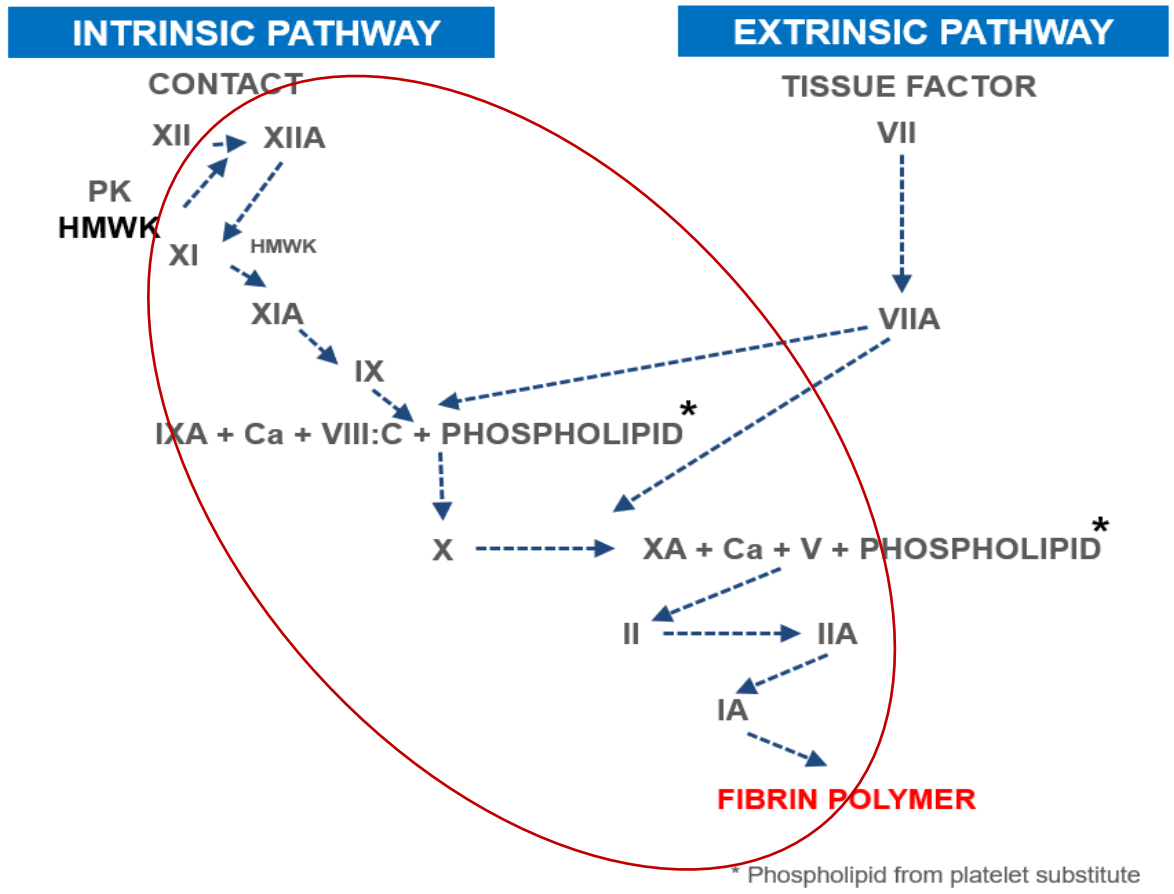
aPTT

Time taken by a recalcified citrated plasma to clot in presence of negatively charged

- Contact **activator** (Silica, Kaolin, Ellagic acid)
- Phospholipid (**Partial Thromboplastin**)

Tests the **Intrinsic Pathway**

- Contact activator will activate the intrinsic pathway by activating FXII onwards with the help of phospholipids



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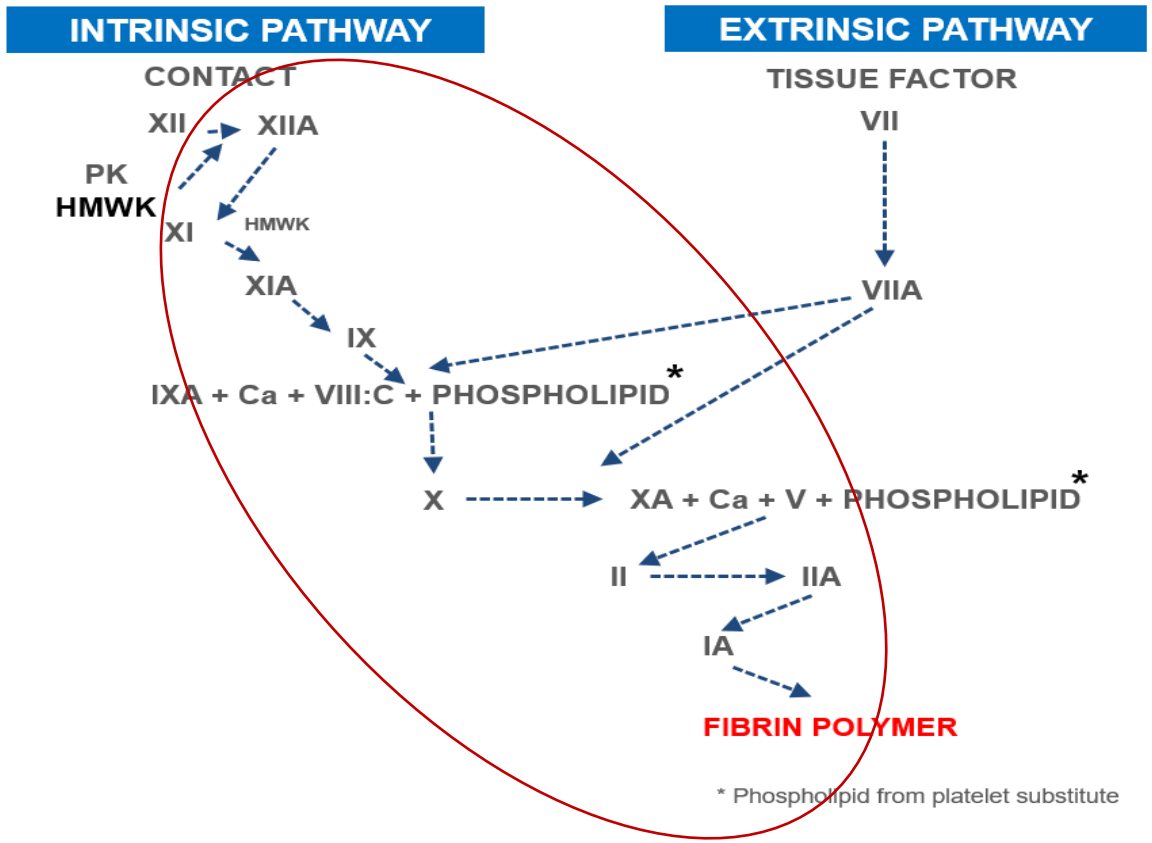
RESULTS



Normal range – 27-37 Seconds

(Different for different reagents)

RESULTING

- Time with Ref Range
- Ratio = $\frac{\text{Patient time}}{\text{Control time}}$
  - >1.2 (6 Seconds more than Control)

REACTIONS INVOLVED IN APT-TIME

PROLONGED aPTT: DEFICIENCY OF FXII,FXI,FIX, FX,FV, FII AND FIBRINOGEN

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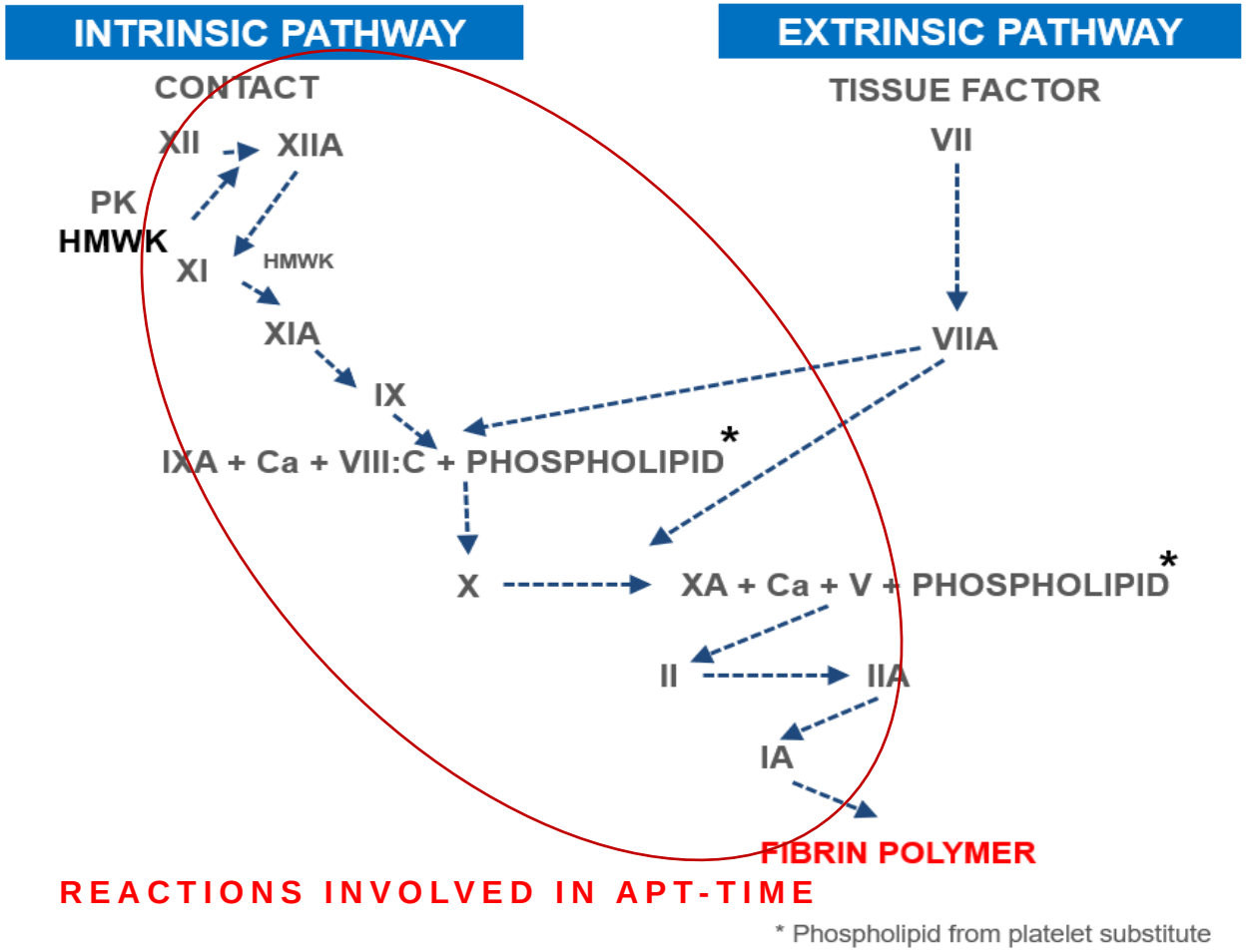
PROTHROMBIN TIME

Time taken by a **recalcified** citrated plasma to clot in presence of Tissue Factor.

Tests the **Extrinsic Pathway**: Tissue Factor will activate the extrinsic pathway by activating FVII onwards.

Tissue Factor - **Thromboplastin** with its bound negatively charged phospholipid.



REACTIONS INVOLVED IN APT-TIME

* Phospholipid from platelet substitute

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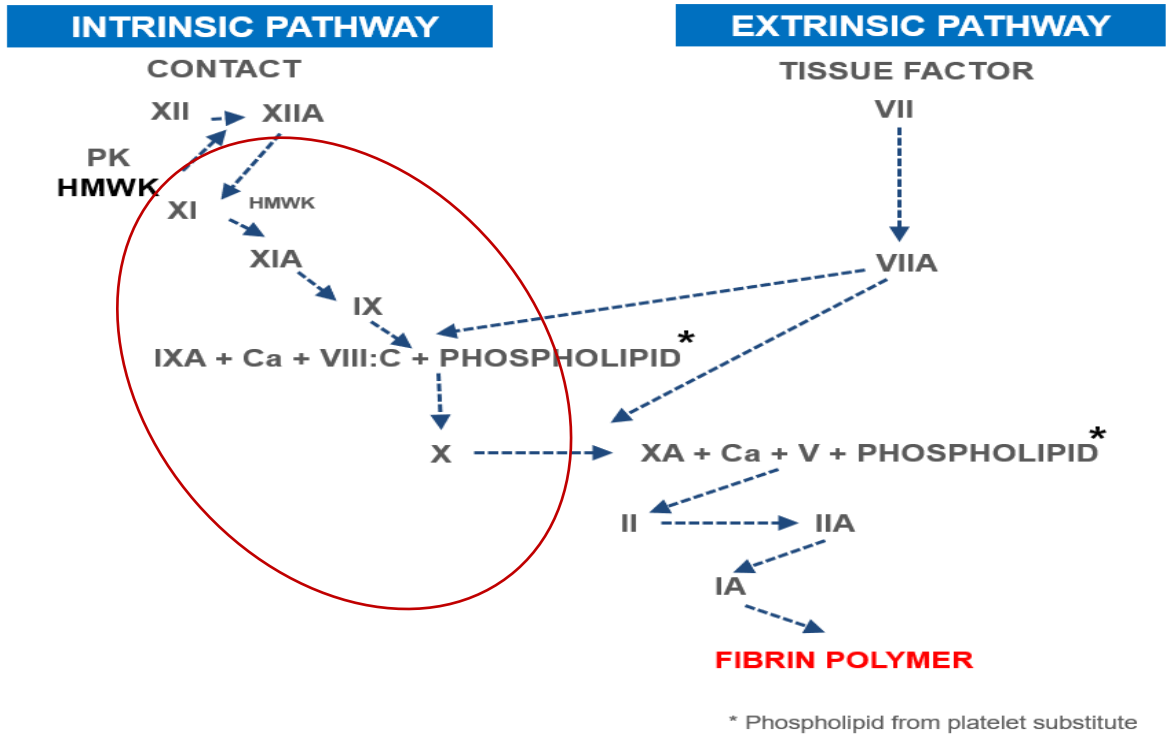
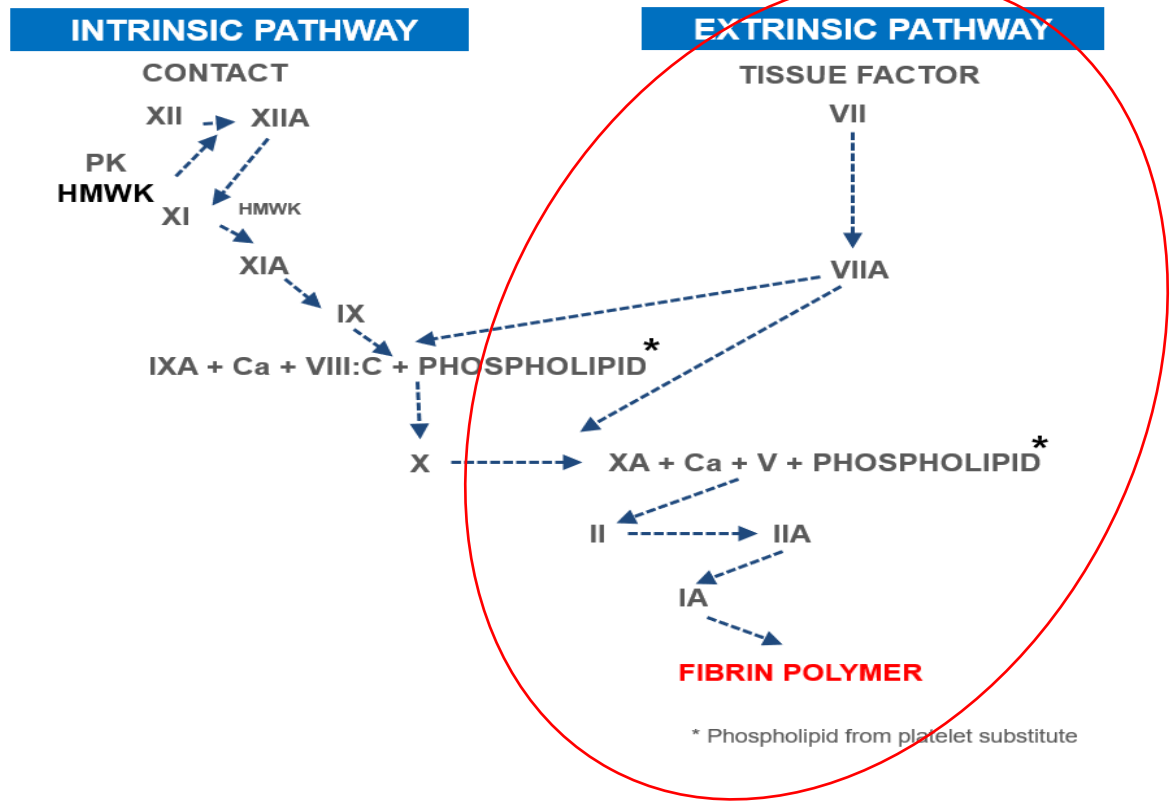
Critical for Haemophilia - APTT

- APTT



- PT



PT – for monitoring Vitamin K antagonist by INR – is a more frequently requested than APTT

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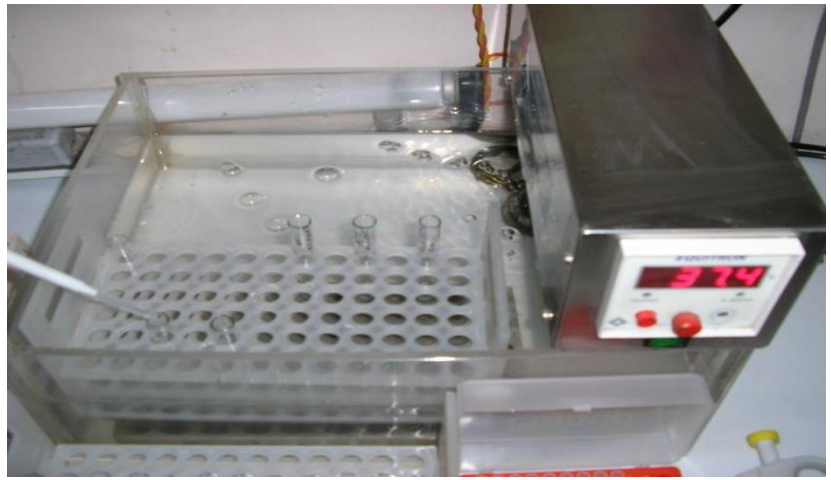
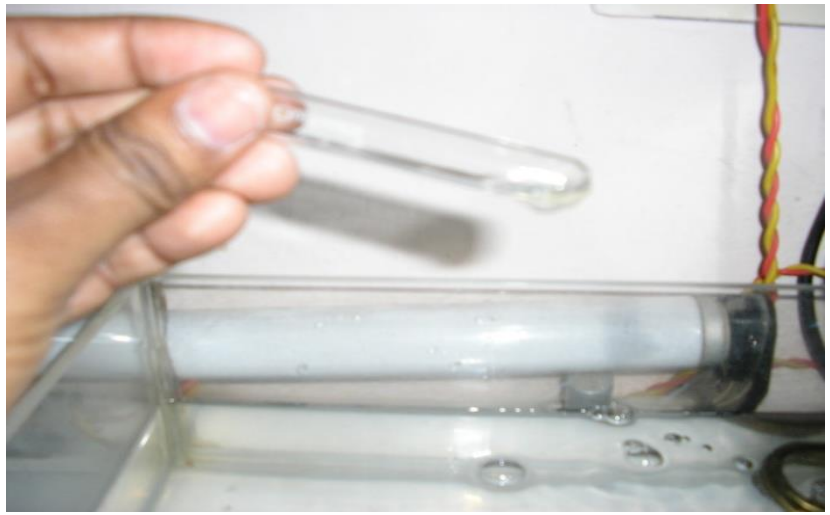
PLASMA BASED TESTS – PT/INR OR APTT MANUAL TUBE TILT METHOD





When PT tests reach 20/month – Semi-automated equipment
Is it difficult to reach this figure?

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IT IS A CHALLENGE

LOGISTICAL CONSTRAINTS:

- Trained Personnel
- Reagents
- Good Sample collection
- Less referrals - testing
- Personnel – motivated to develop non-mandated lab procedure

LACK OF REAGENTS:

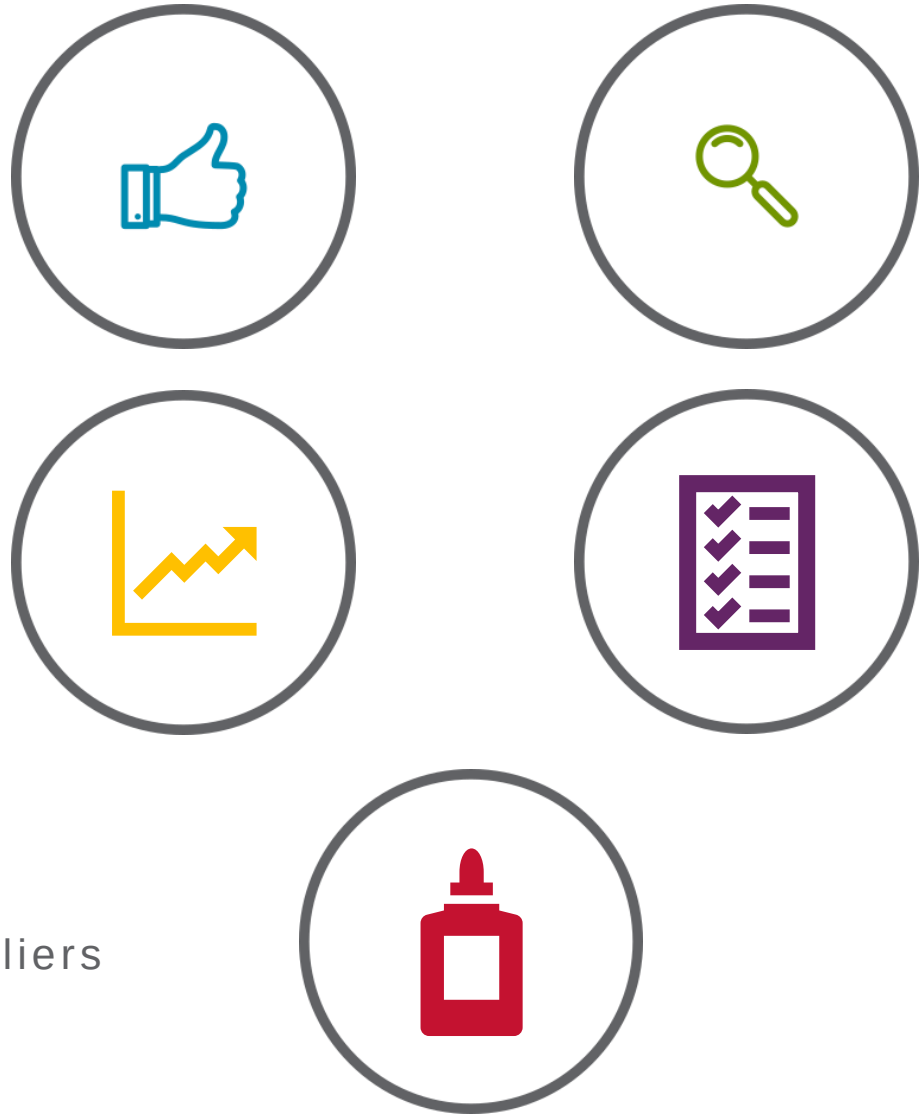
- No supply or supplier or is not regular
- Cost
- Short shelf life
- Short window within which most of the tests/assays have to be done,
- Stringent issues of sample and reagent storage

STRATEGIES TO OVERCOME VIS-À-VIS REAGENTS

- Make do with what is available
- Maximize supply and Minimize wastage
- Simple storage solution and In-house reagents

- Increase referrals
- More tests → more consumption → busy and increased burden of work → automation

- Brings in suppliers



- Source simple material and instrumentation
- Simple but stringent storage solutions with continuous power

- Increase reliability and Reduce risk of failure of discontinuation of a test
- Good methodology and simple but strict IQC → gaining the trust of the referring physician

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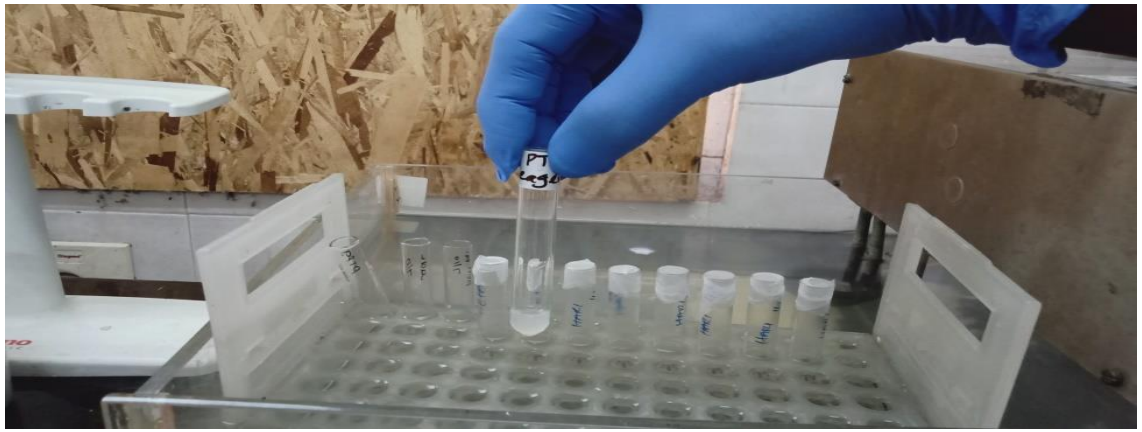
SCREENING TESTS IN LOW-RESOURCE ENVIRONMENT

- PT/INR and APTT
- Choice of good reagents
  - Not an issue
- Frequency of testing
  - Is an issue
    - One per week/month to more than one per day
- Thromboplastin Shelf life and volume
  - One week
  - Not less than 3 mL (3000 µL)
    - 1200 µL for manual method and 300 µL for semi-automated

THROMBOPLASTIN REAGENT - MAXIMIZE ON IT AND MINIMIZE WASTAGE



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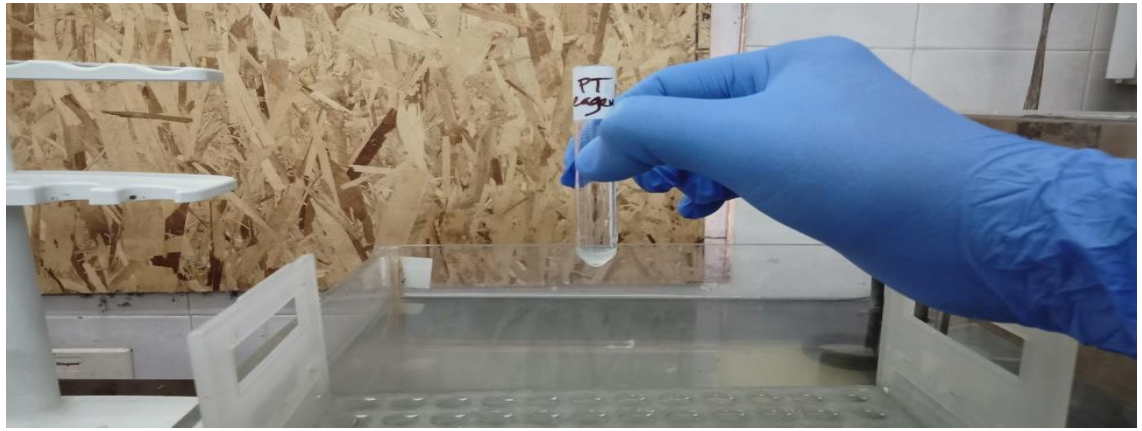
THROMBOPLASTIN REAGENT - MAXIMIZE ON IT AND MINIMIZE WASTAGE



BLEEDERS HEMOPHILIA

- APTT
- Mixing studies
- Factor assays
- Inhibitors

- Infrequent referrals – once a week or once a month
- APTT Reagent – same strategy as PT/INR
  - Selection – must have high sensitivity to Factor Deficiency, moderate sensitivity to Heparin levels and low sensitivity for Lupus anticoagulant

CORRECTION STUDIES BY MIXING

Differentiate if a severe bleeding patient has factor deficiency or not when the test is repeated on the mixture of patient's plasma and normal plasma

- Normal pool plasma (where all factors are present in normal quantity) – Pooled Normal Plasma (PNP)
- Which factor is the deficient factor
  - Is it FVIII (Haemophilia A) or
  - FIX (Haemophilia B)

PLASMA/REAGENTS USED FOR MIXING STUDIES

Selective plasmas (Plasma deficient in some factors)

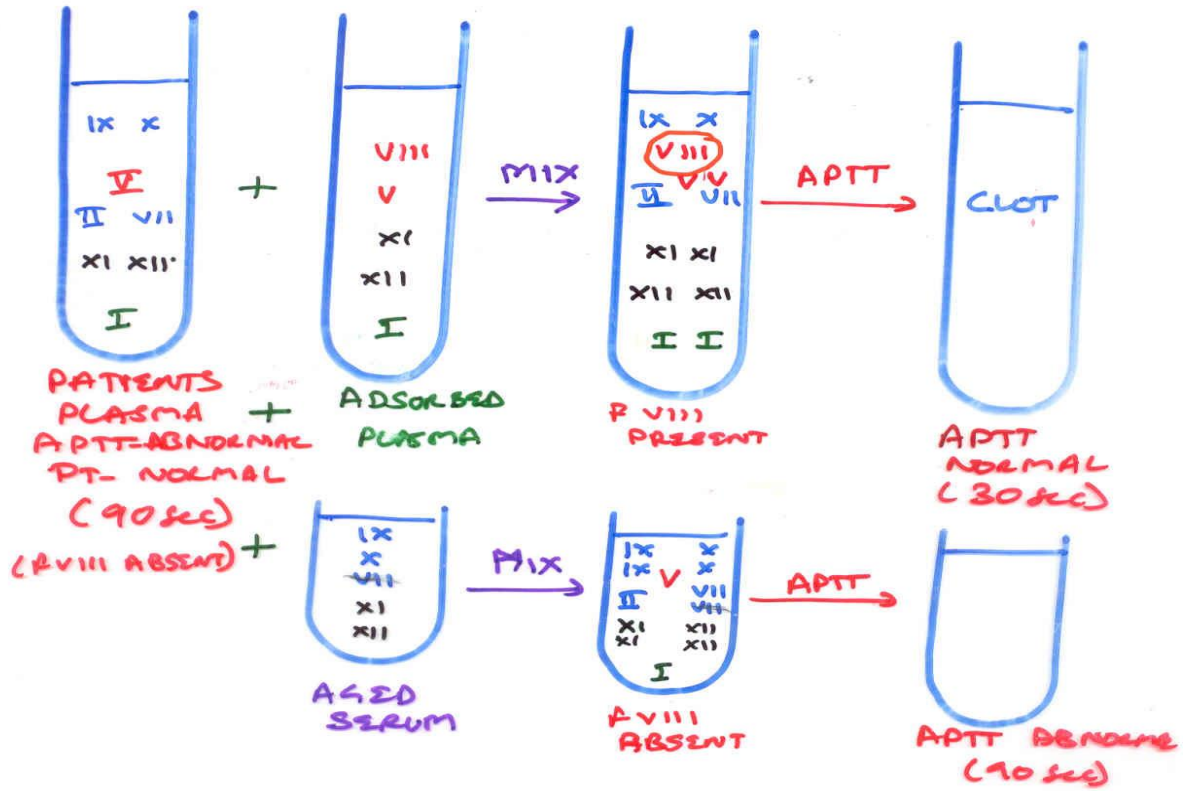
- Patient derived
  - *Haemophilia A- Deficient in Factor 8
  - *Haemophilia B- Deficient in Factor 9

Contrived/artificial (this can be produced from normal blood sample)

- **Adsorbed plasma** - adsorb out Vit K dependent factors with Cat ions (Ba^{++})
  - Contains only F VIII, FV, FXII, FXI and Fibrinogen
- **Aged Serum** – from blood collected in a clot tube from a normal donor which has been kept over 48-72 hours
  - FII and Fibrinogen is lost in the clot and FVIII and FV are lost in time – FIXa, FXa, FXIa
- - Aged Serum **Always available in any laboratory**
- **- Pick up 3 each from different normal individual's tubes**

EXAMPLE

HAEMOPHILIA - 'A' - FACTOR VIII DEFICIENCY



CORRECTION WITH ADSORBED PLASMA

NO CORRECTION WITH AGED SERUM

DIAGNOSIS: FACTOR VIII DEFICIENCY

CORRECTION STUDIES BY REPLACEMENT

- Using FVIII concentrate: a patient is in your centre with bleeding and has a prolonged APTT and mixing studies with PNP show good correction of APTT

Take a **used vial** of FVIII concentrate. Note the dose. It is usually 100 U/mL

↓

Dilute it 1:4 with normal saline or distilled water

↓

Add 6 µL of the diluted FVIII concentrate to 150 µL of the patient plasma

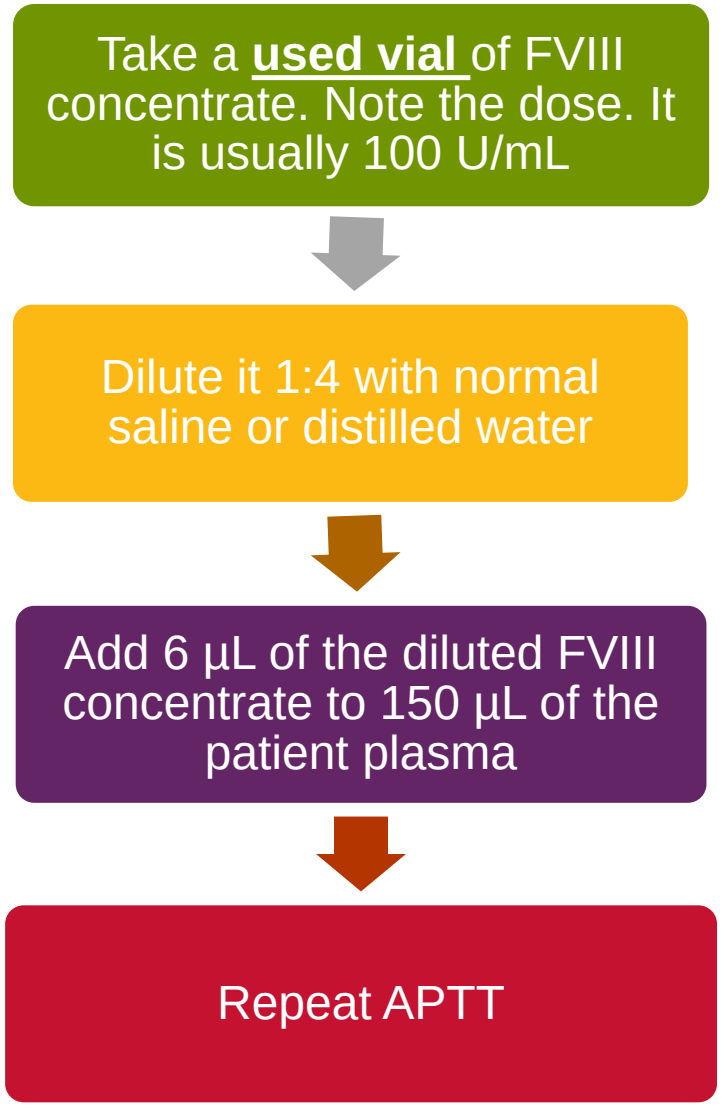
↓

Repeat APTT

CORRECTION STUDIES BY REPLACEMENT

- If the APTT is corrected it is FVIII deficiency
- If the APTT remains abnormal it is FIX deficiency
- Or the sample has high levels of FVIII inhibitors

- Inhibitor screen
- FVIII Assay
- Both are a form of Mixing studies using materials discussed so far



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FACTOR ASSAY REAGENTS

- Normal reference plasma : to draw std graph –
- commercial (traceable to SSC/ISTH Std)
- in house (PNP – assigned values traceable to a calibrator).
- Control plasma: Normal & abnormal
- Factor deficient plasma: (contents will determine the time)
- Imidazole buffer (Owren’s veronal buffer may be used; pH: 7.35-7.4).
- CaCl2.
- APTT reagent.
- Cost and Shelf life

STORING **REFERENCE** AND DEFICIENT PLASMA

- Requiring a Freezer
  - –80oC for up to 1 year
    ≈ USD 10,000 – 15,000
  - –20oC for up to 1 month
    ≈ USD 3,000 – 4,000

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SIMPLE BUT STRINGENT STORAGE SOLUTIONS WITH CONTINUOUS POWER

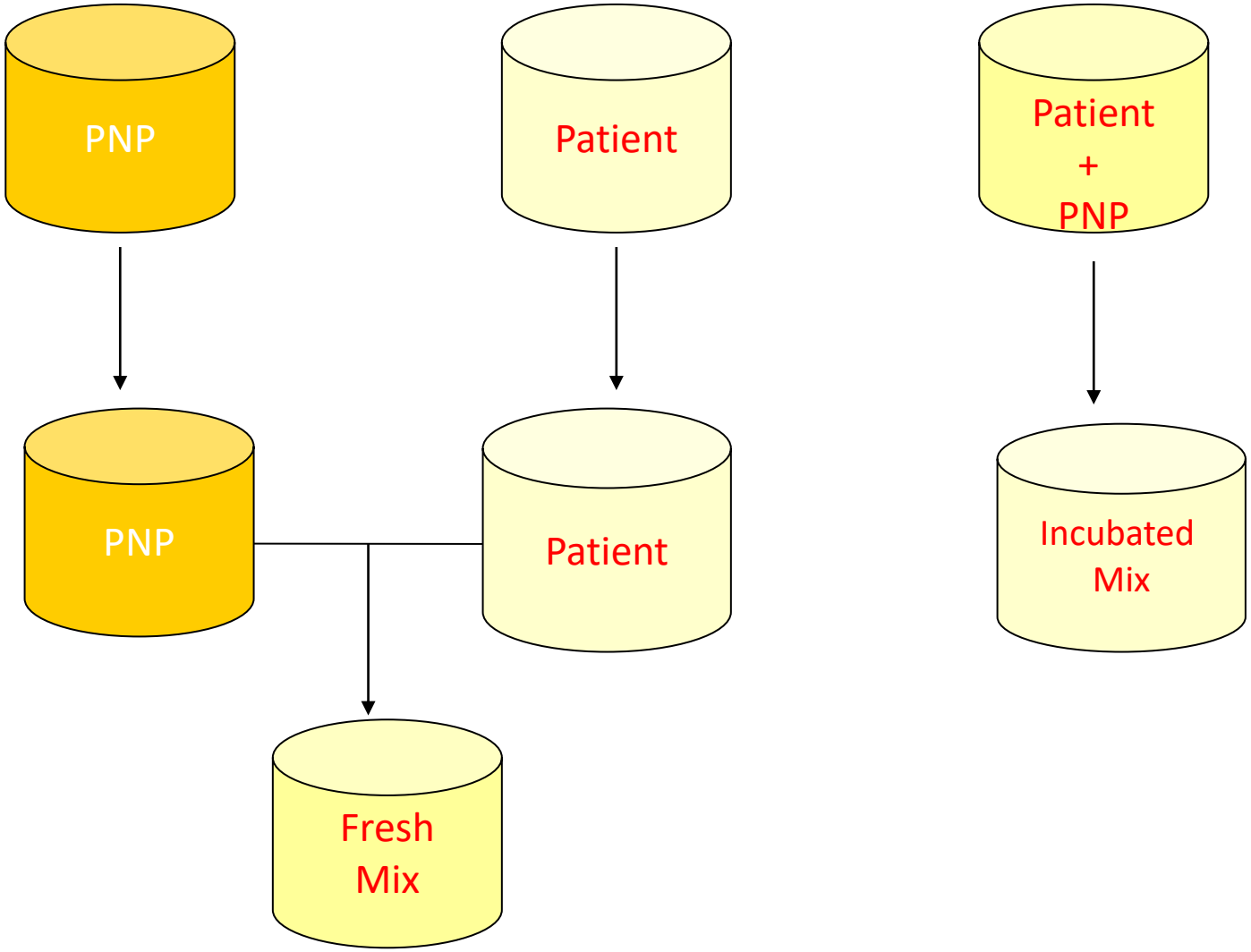
- Domestic Freezers
  - -18^{0}C to -22^{0}C
- USD – 250.
- Stand alone with UPS
- Not freezers of domestic Fridge
  - Freeze thaw cycles for defrost
- Less time outside the freezer
  - Stability noticed up to 3 months

INCREASE RELIABILITY AND REDUCE RISK OF FAILURE WITH STRICT IQC

- Controls
  - 2 levels
- PT/INR & APTT
  - Normal - PNP
  - Abnormal –
    - Harvest remaining plasma from INR 2.0-2.5 into a vial and box it in a freezer
    - When 20-30 – thaw them, pool, aliqout and store for daily use
- Factor assay
  - Mix PNP with Factor Deficient plasma ≈ 20%

Once Volume goes up – shift to commercial reagents

INHIBITOR SCREENING - APTTS OF :



APTT (sec) of Incubated – fresh mix > 10 sec: Inhibitor +

You can improve the sensitivity of this test by using a normal plasma with 50-60% FVIII and a responsive APTT reagent

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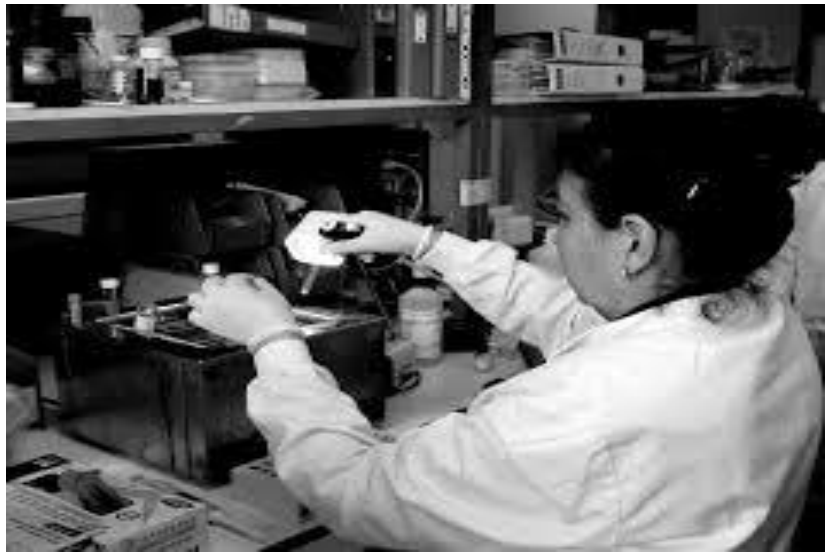
WFH INTERNATIONAL EXTERNAL QUALITY ASSESSMENT SCHEME (IEQAS) PROGRAM



- Whatever the numbers, but for all established tests participation in a Proficiency testing program is a must.

PERSONNEL

MOTIVATED TO DEVELOP NON-MANDATED LAB PROCEDURE

- Not common
- Mandatory
- Transfusion services
  - Haemorrhagic conditions
  - PPH (Post Partum haemorrhage)
    - Fibrinogen is mandatory
    - Will require standard reagent. Difficult in-house
- Trauma
- QC of products (FFP and Cryo)
  - Fibrinogen
  - FVIII
  - APTT

THANK YOU!

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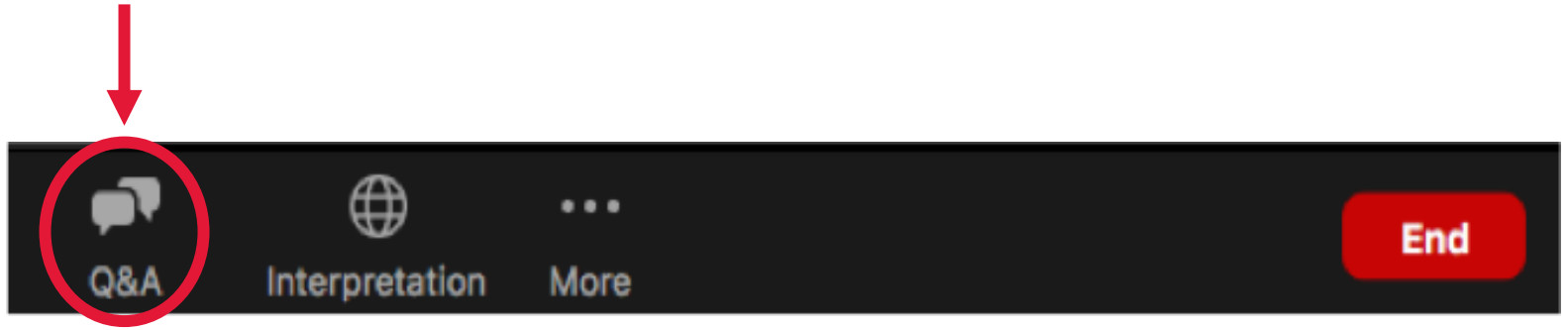
QUESTION & ANSWER

Please submit your questions in the Q&A box



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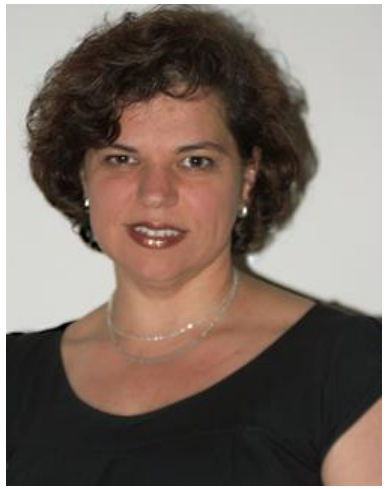
SPEAKERS



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Chromogenic Assays: Why are they useful in hemophilia

Margareth Ozelo, MD PhD

Hemocentro UNICAMP, Brazil

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Margareth Ozelo

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Speaker bureau	BioMarin, Novo Nordisk, Roche, Takeda

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Measuring factor VIII or factor IX activity

When is clinically important?

- For diagnosis
  - Severity assessment
- For monitor FVIII or FIX therapy
  - Prophylaxis for interventions (surgeries)
  - Apparent resistance to factor replacement
  - For better prophylaxis outcomes:
    - pharmacokinetic determinants (eg. peaks and trough levels)
- As a step of others laboratory analysis
  - To evaluate the presence of neutralizing antibodies (inhibitors)
    - Bethesda-Nijmegen assay

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Kitchen *et al.* Haemophilia 2016 Jul;22Suppl 5:72-7

Measuring factor VIII or factor IX activity

How to measure factor VIII and IX activity?

- One-stage (aPTT-based) assay
  - Simple and easy to perform, and more commonly used for monitoring
- Two-stage (IIa generation) assay
  - Uncommonly used (replaced by chromogenic assay)
- Chromogenic (Xa generation) assay

aPTT = activated partial thromboplastin time

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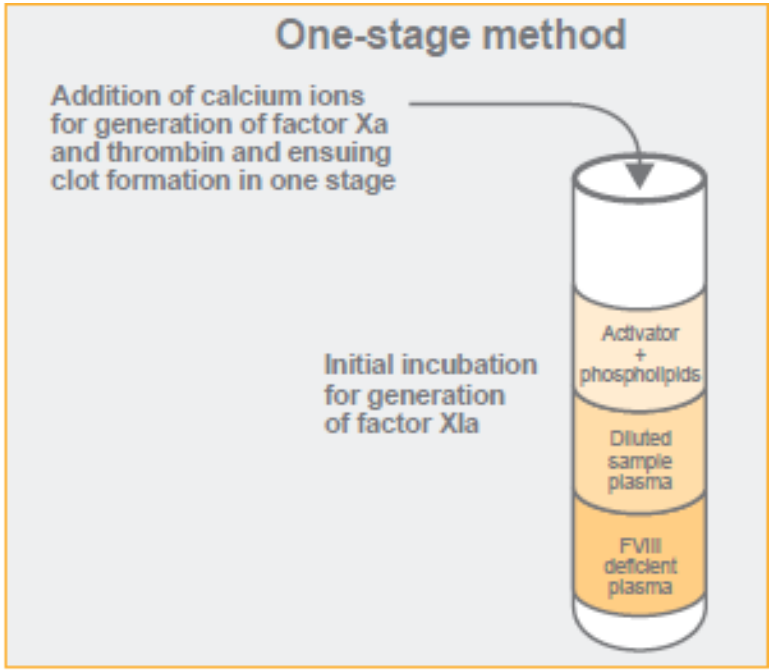
Kitchen *et al.* Haemophilia 2016 Jul;22Suppl 5:72-7

One-stage FVIII/FIX clotting assay - aPTT

- Initiation of coagulation, FVIII activation, and subsequent FXa, thrombin and fibrin generation occurs in a “single reaction” thus termed “one-stage”
- FDA recommends the one-stage clotting assay to assign FVIII and FIX concentrate potency

aPTT in diluted patient sample with FVIII/IX deficient plasma



- FVIII-deficient plasma (+/- VWF) or FIX-deficient plasma
- Phospholipid
  - Total amount/PS content
- Contact activator
  - Ellagic acid/kaolin/micronised silica

❖ Timer starts with the addition of calcium to initiate the reaction and stops when reaches a preset increase in one of the following:
  - Turbidity (optical measurement)
  - Viscosity (mechanical measurement)

Endpoint: Clot time

aPTT = activated partial thromboplastin time
VWF = von Willebrand factor; PS = phosphatidylserine

Gu *et al.* Haemophilia. 2014; 20. 593-600
Malar et al., Eur J Haematol. 2020;104:3–14

One-stage FVIII/FIX clotting assay - aPTT

Activator	Phospholipid	Calibrators
Ellagic acid based		
Ellagic acid	Synthetic	Plasma derived standards
Polyphenolic acid	Cephalin	Chromogenic standards
Silica based		
Colloidal silica	Soya	Concentrate specific standard
Micronized silica	Vegetable/Plant	
Silica dioxid	Bovine	
Sulfatides and silica	Rabbit brain	
Kaolin	Porcine/chicken	

aPTT = activated partial thromboplastin time

Gu *et al.* Haemophilia. 2014; 20. 593-600

One-stage FVIII/FIX clotting assay - aPTT

Limitations

- Considerable inter- and intra laboratory variation due to
  - Different instrument platforms
  - Choice and handling of reference material
  - Misleading when assaying for potency of rFVlll or rFIX products

- Large variability due to
  - Sources of clotting activators, phospholipids and factor deficient plasma may differ, kaolin activators are too dense for optical analyzers
  - Susceptible to interference from preactivation of FVlll or FIX generated during venipuncture
  - Interference from anti-phospholipid antibodies

aPTT = activated partial thromboplastin time

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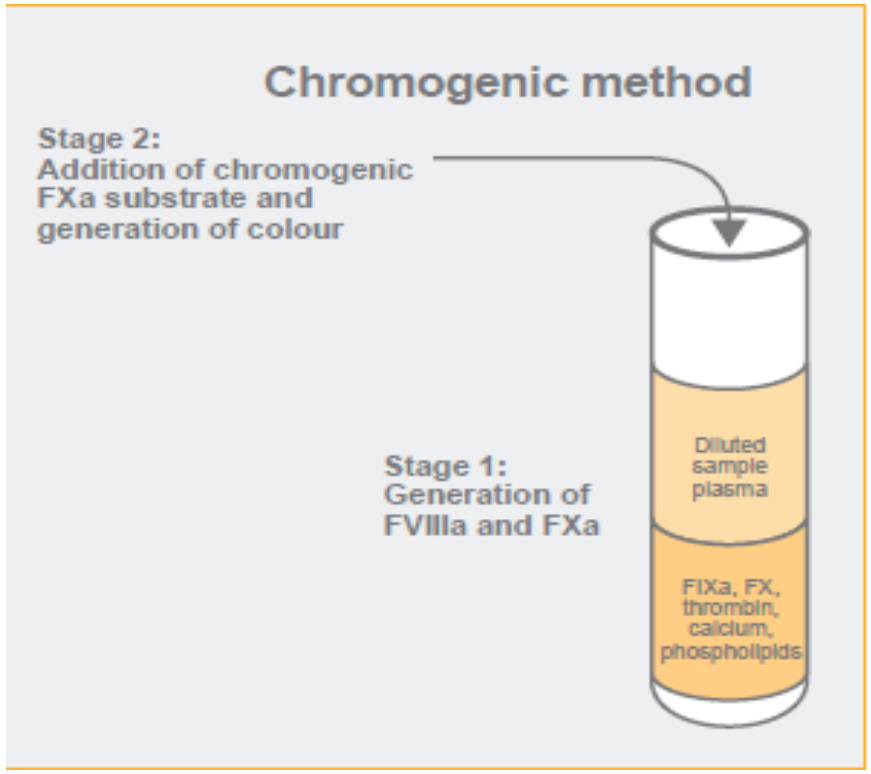
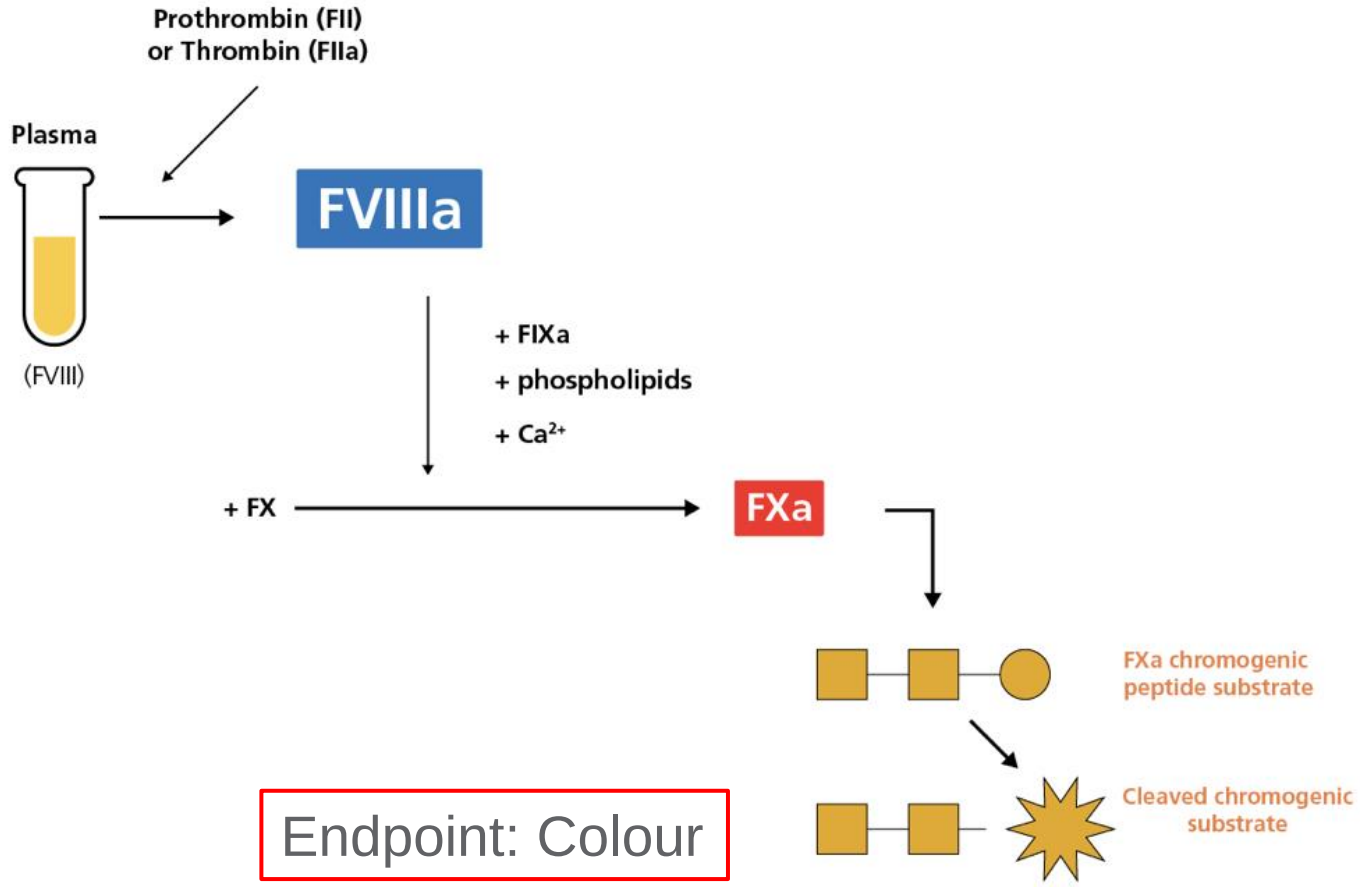
Gu *et al.* Haemophilia. 2014; 20. 593-600

Chromogenic FVIII assay

- Direct determination of FVIII cofactor activity: FVIII mediates FXa generation
- The European Pharmacopoeia (EMA) recommends use of the chromogenic-based assay to assign FVIII concentrate potency





www.coagulationassays.com

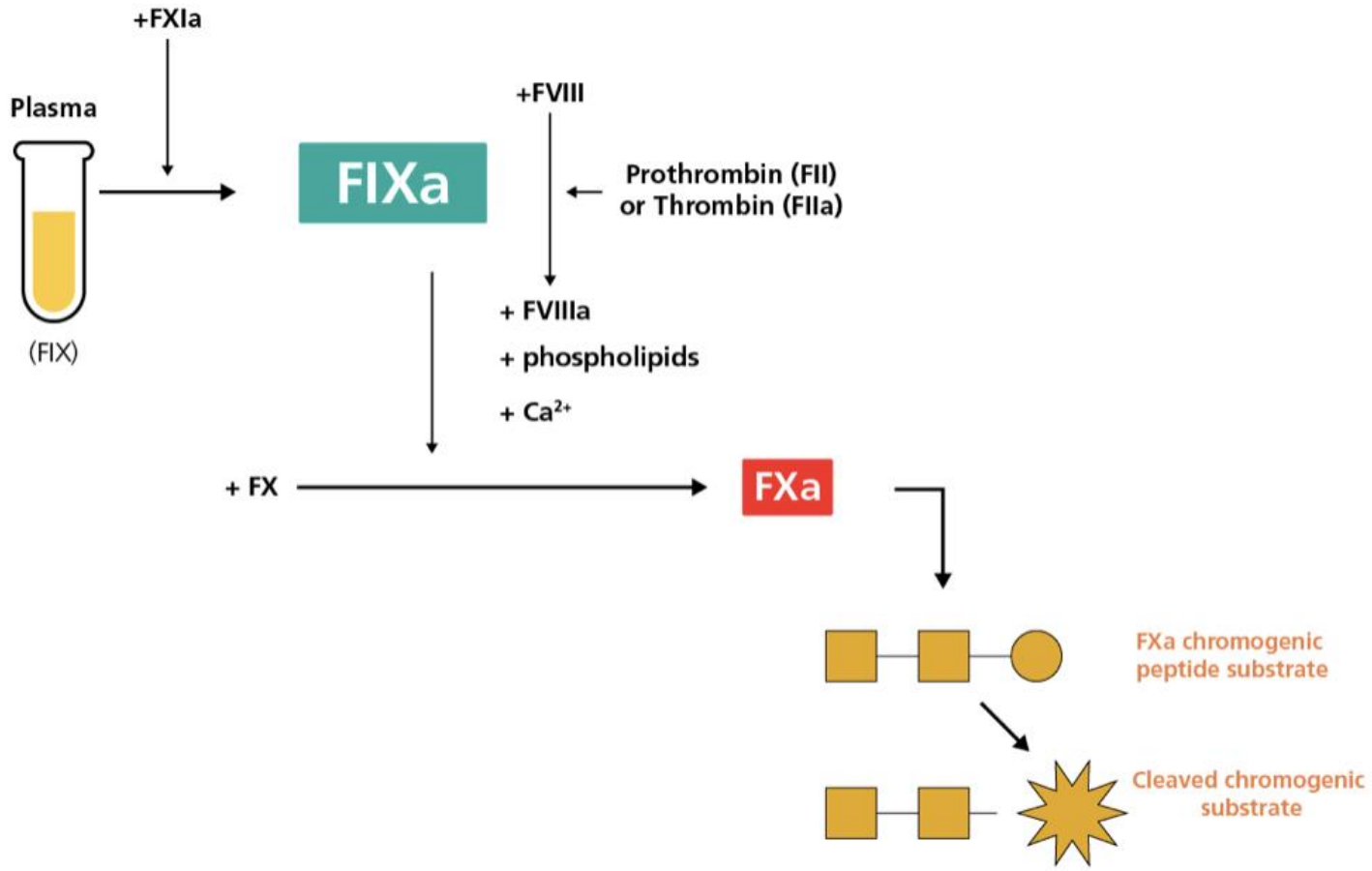
Malar et al., Eur J Haematol. 2020;104:3–14

Chromogenic FVIII assay

Chromogenic FVIII assay	FIXa and FX reagents	Manufacturer
Chromogenix kit		
Coamatic Factor VIII Coatest Factor VIII Coatest VIII:C/4	Bovine	Chromogenix a brand of Instrumentation Laboratory (US)
HemosIL Electrachrome Factor VIII	Bovine	Instrumentation Laboratory (US)
Factor VIII Chromogenic Assay	Bovine	Siemens Healthcare (Germany)
Technochrom FVIII:C	Bovine FX Human FIXa, IIa	Technoclone (Austria)
Biophen FVIII:C	Human	Hyphen BioMed (France)

Chromogenic FIX assay

- FIX is activated by human FXIa with concomitant activation of human FX by generated FIXa
  - in the presence of FVIII, calcium ions and phospholipids



Chromogenic FIX assay

Chromogenic FIX assay	Reagents	Manufacturer
ROX FACTOR IX	Reagent A: human FVIII, human FX, bovine FV Reagent B: human FXIa, human FII, calcium chloride and phospholipids	Rossix AB (Sweden)
CH9 Chromogenic Factor IX Activity Assay		Mayo Medical Laboratories (US)
BIOPHEN™ FIX	Reagent 1: human FX and human FVIII Reagent 2: human FXIa, human thrombin, calcium chloride, imidazole, synthetic phospholipids	Hyphen BioMed (France)

Factor VIII activity for SHL or EHL FVIII concentrates

Monitoring SHL FVIII concentrates

- Differences according to OSA reagents, type of rFVIII products, and standards used
  - Full-length rFVIII in plasma may result higher level when measured by CSA with plasma standards used for calibration
  - B-domain deleted rFVIII (BDD-rFVIII) usually shows higher levels when measured by OSA
- Chromogenic assays usually show better laboratory agreements in field studies

Monitoring EHL FVIII concentrates

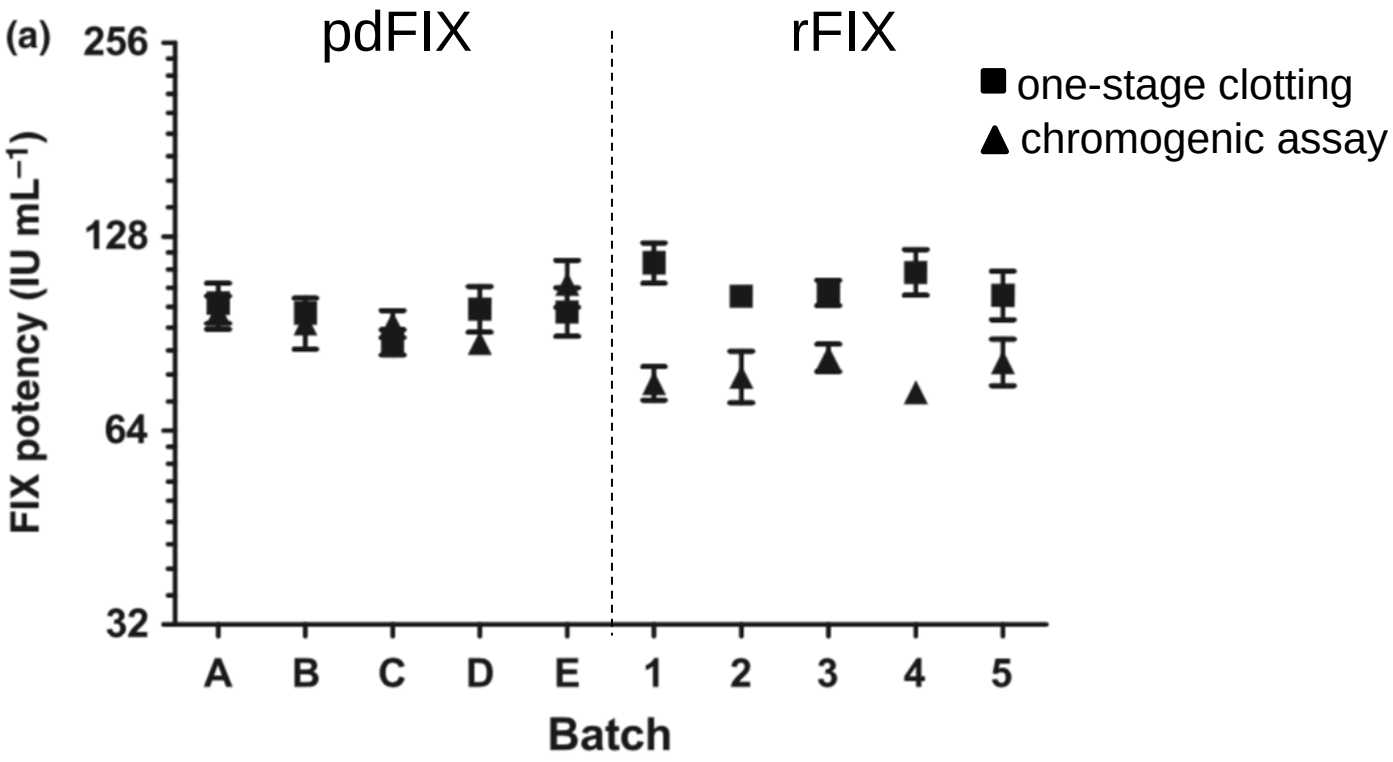
Reagent name	N8 GP (Esperoct®)	rFVIIIFc (Eloctate®)	BAY rFVIII-PEG (Jivi®)
Chromogenic assay	Yes	Yes	Yes
One-stage reagents			
STA-PTT A	No	Yes	No
STA-C.K. Prest	Yes	Yes	?
Actin FS	Yes	Yes	?
Actin FSL	Yes	Yes	?
Pathromtin SL	?	Yes	?
SynthASil	?	Yes	?
SynthAFax	No	?	Yes
DG Synth	Yes	?	?
Cephascreen	Yes	Yes	Yes
APTT–SP	No	?	No

SHL, standard half-life; EHL, extended half-life
OSA, one stage assay; CSA, chromogenic substrate assay

Kitchen et al. Haemophilia 2014;20 (4), 36–42
Kitchen et al. Semin TH. 2017;43(3):331-7

Factor IX activity for SHL FIX concentrates

Chromogenic *vs.* One-stage FIX assays



- Chromogenic FIX activity is around 70% of one-stage results for rFIX concentrates
- One-stage FIX activity can be affected by the reagents used

SHL, standard half-life
pdFIX, plasma-derived FIX; rFIX, recombinant FIX

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Wilmot *et al.* Haemophilia 2014, 20, 891-7
Kitchen *et al.* Haemophilia 2016 Jul;22Suppl 5:72-7

Factor IX activity for EHL FIX concentrates

Reagent name	N9 GP (Rebinyn/Refixia®)	rFIXFc (Alprolix®)	FIX-Albumin (Idelvion®)
Chromogenic FIX assay	Yes	Yes	Yes
One-stage reagents			
STA-PTT A	No	Yes	Yes
STA-C.K. Prest	No	No	No
Actin	No	?	?
Actin FS	No	Yes	No
Actin FSL	No	Yes	?
Pathromtin SL	No	Yes	Yes
SynthASil	No	Yes	Yes
SynthAFax	Yes	?	?
DG Synth	Yes	?	?
Cephascreen	Yes	Yes	?
APTT–SP	No	?	?
Auto APTT	?	Yes	?
Platelin L	?	Yes	?
DAPTIN	Yes	?	?

EHL, extended half-life

Assays that can be used to monitor selected EHL FIX products

"Yes" means results were within 25–30% of the expected result based on labeled potency

"No" means results were more than 30% different from expected

"?" means authors are unaware of any data at the time of writing this article

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Kitchen *et al.* Semin TH. 2017 Apr;43(3):331-7
Malar et al., Eur J Haematol. 2020;104:3–14

Assay discrepancies for endogenous FVIII or FIX

Non-severe hemophilia

Non-severe hemophilia A	
OSA > CSA	OSA < CSA
F8 mutations in A1-A2-A3 domain (destabilization of the FVIIIa heterotrimer)	*F8* mutations in VWF or FIXa binding sites, or thrombin cleavage sites (supraphysiological factor concentrations in CSA)

- **Recommendation:**
  - Consider the bleeding phenotype for severity assignment
  - Patients with non-severe hemophilia A, both OSA and CSA should be performed
  - Not enough information for hemophilia B

OSA, one stage assay; CSA, chromogenic substrate assay

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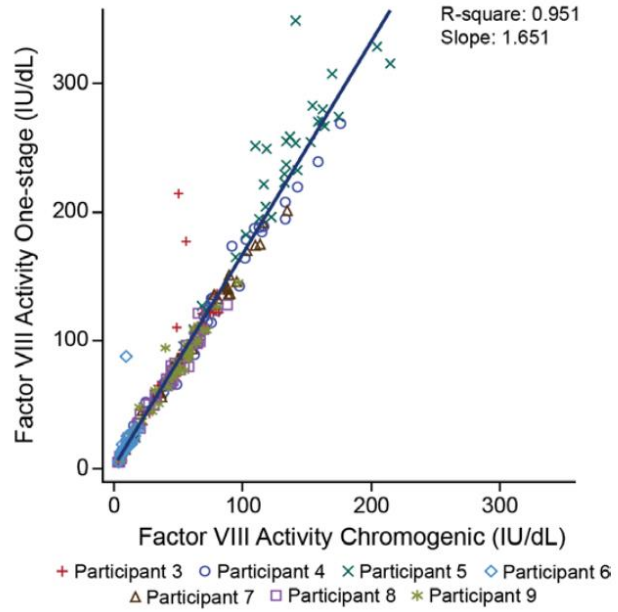
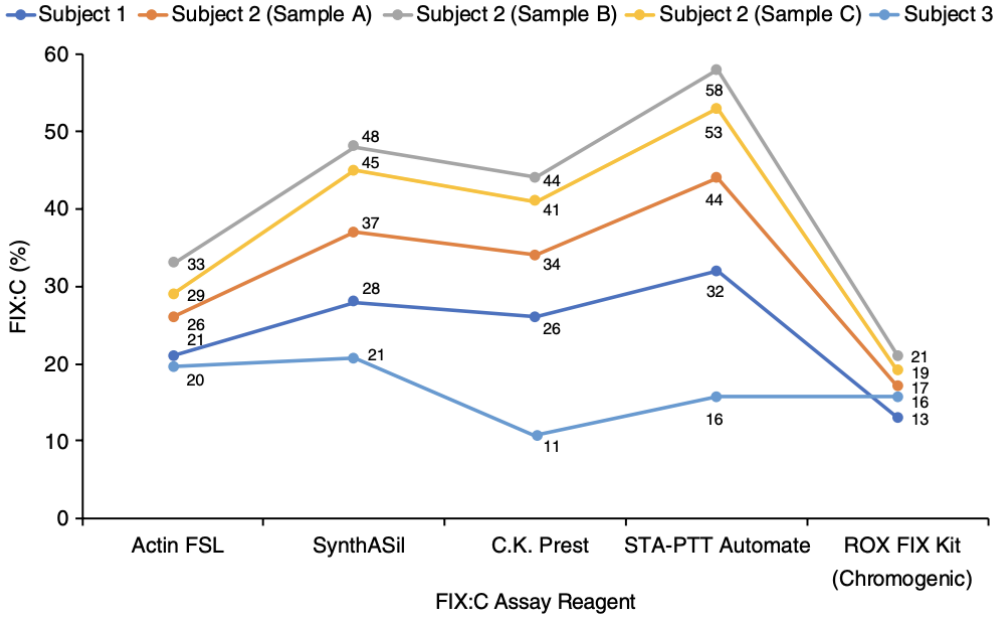
Kitchen *et al.* Haemophilia 2016 Jul;22Suppl 5:72-7

Assay discrepancies for endogenous FVIII or FIX

Gene therapy

Hemophilia A	Hemophilia B
BDD-FVIII gene product	FIX-Padua gene product
Chromogenic FVIII activity is 60% less than one stage[1,2] (opposite for BDD-rFVIII Refacto AF®)	Chromogenic FIX activity is 60% less than one-stage[3] One stage vary according to reagents[4]





BDD-FVIII, B-domain deleted factor VIII

1. Rangarajan et al. N Engl J Med 2017;377:2519-30
2. Rosen et al., Blood 2020;136:2524-34
3. George et al., N Engl J Med 2017; 377:2215-27
4. Robinson et al., J Thromb Haemost. 2021;00:1–7

FVIII assay in presence of emicizumab

- Emicizumab interferes with clotting-based assays, such as the aPTT and one-stage assays
- **Recommendation:**
  - For **FVIII activity measurement and to quantify FVIII inhibitor** in presence of emicizumab the recommendation is to use of the **chromogenic method containing bovine proteins**
  - To evaluate the **expected emicizumab levels**, it is possible to use of the **modified one stage assay method**, including an additional pre-dilution and calibration of the assay with an emicizumab-specific calibrator

aPTT = activated partial thromboplastin time

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Malar et al., Eur J Haematol. 2020;104:3–14
Srivastava et al., Haemophilia 2020 Aug;26 Suppl 6:1-158

In Summary

- Chromogenic assays may avoid some limitations associated with one-stage assays, but their regulatory status, higher cost, and lack of laboratory expertise may influence their use
- For factor VIII and IX monitoring
  - Chromogenic assay suitable for monitoring modified and unmodified FVIII and FIX
  - **Laboratories** should have knowledge about which replacement factor the patient is receiving and if their current method provides an accurate measurement
  - **Clinicians** must be informed about the potential biases (over- or underestimating factor activity) and an alternative of measuring accurate concentration provided

Thank you!

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Bleeding Disorder Products: The long journey of production

Radek Kaczmarek, PhD

Chair WFH CPSSA Committee

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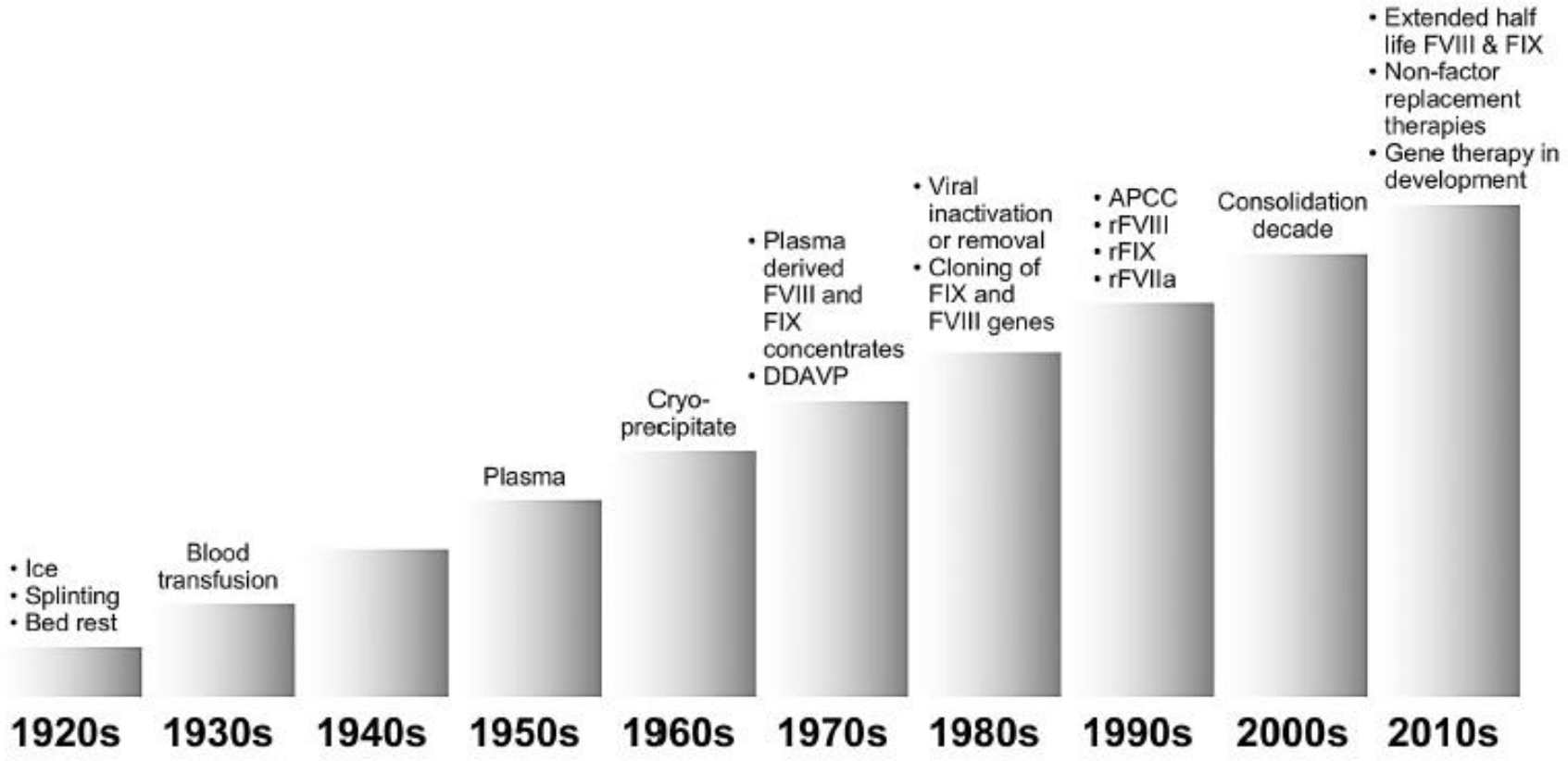

Evolution of hemophilia therapy: whole blood -> plasma-> cryoprecipitate -> pdCFCs -> rCFCs -> EHL rCFCs -> non-factor products -> gene therapy

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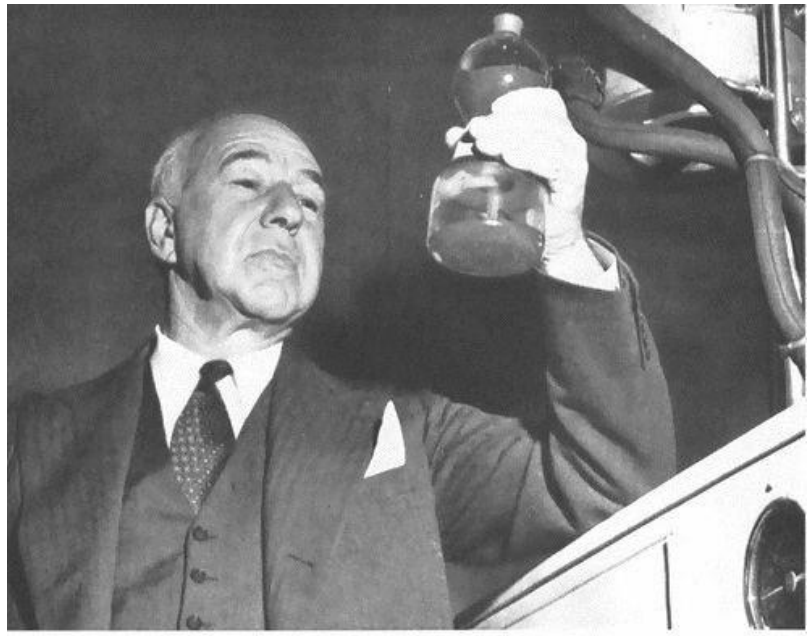
Manucci PM, Haematologica, 105(3), 545-553, 2020

Key early technological leaps (1)



Cold ethanol fractionation and purification of albumin.

Proof of concept for large scale isolation of therapeutic proteins from plasma (1940s).

Cohn et al, J Am Chem Soc, 68(3), 459–475, 1946

Key early technological leaps (2)

Discovery of high FVIII:C activity in cryoprecipitate.

Cryoprecipitation results from slow thawing of whole plasma at 1 to 4ºC.

Cryoprecipitate consists of FVIII, VWF, fibrinogen and FXIII.

Still used in the developing world for treatment of hemophilia; fills a gap between patients' needs and affordability.

Judith Graham Pool

Can be made at local blood banks.

Usually not virally inactivated (risk of blood-borne infections).

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Pool JG et al, Nature, 203, 312, 1964

Plasma fractionation today (1)

Table 2. Typical Plasma Protein Downstream Purification Methods

Method	Description	Separation principle	Application
Cryoprecipitation	Thawing of whole plasma; at +1°C to +4°C	Differential solubility at cold positive temperature	Precipitation of FVIII, VWF, and fibrinogen
Ethanol precipitation	Successive precipitation steps of cryo-poor plasma by Ethanol (10%-40%), under precise conditions of pH (ca 7.4-4.5), temperature (−3 to −6°C), protein concentration, and ionic strength Removal of precipitates by centrifugation or depth-filtration	Differential solubility in ethanol at cold negative temperature	Precipitation of fibrinogen, IgG, albumin, AAT, etc
Ion exchange chromatography	Binding of proteins on a solid support usually packed in a column. Can also be done as a batch process, eg, DEAE, QAE, CM...	Electric charge binding. Elution by increasing salt content or changing pH	Most coagulation factors, protease inhibitors, and anticoagulants
Affinity chromatography	Binding of proteins on a solid support most usually packed in a column; ligands include heparin, metals, and gelatin	Specific affinity ligand proteins. Elution usually by increasing salt content	AT, VWF, FIX, etc
Immunoaffinity	Binding of proteins on a solid support packed in a column. Ligands include murine monoclonal antibodies	Specific affinity antibodies-proteins. Elution usually by increasing salt content	FVIII, FIX, protein C
Size-exclusion chromatography	Injection of proteins on a solid support packed in a column	Separation based on differential molecular mass	AAT, FVIII
Ultrafiltration	Selective fractionation process on membranes of defined pore size that concentrates protein and removes low-molecular-weight solutes and salts	Separation based on differential molecular mass	All products
Microfiltration	Low-pressure cross-flow membrane process for separating colloidal and		All products

Reception of plasma shipped by blood establishments
Control of shipping parameters and reconciliation

Quarantine of plasma in cold room (e.g. <-20°C or colder)
Control of documentation
Additional testing and quality control

Preparation of plasma batch

Conditioning of plasma packs

Pooling and large-scale processing
Opening of the plasma packs
Cryoprecipitation
Bulk fractionation steps (such as ethanol fractionation)
Preparation (and storage) of intermediate fractions
Protein downstream processing and viral reduction treatments of individual proteins
Finishing and terminal sterile filtration steps (0.2µm)

Aseptic dispensing
Filling in vials / bottles
+/- Freeze-drying

Quarantine – quality control

Check of production batch records and documentation

Labelling - Packaging

Boxing - Shipment

Burnouf T, Trans Med Rev, 21(2), 101-117, 2007

Plasma fractionation today (2)

Table 1 Range of plasma products and clinical use [adapted from (4)]

Protein	Clinical use
Fibrinogen	Congenital or acquired (postpartum hemorrhage) deficiency
Factor II	Factor II deficiency
Thrombin (factor IIa)	Component of fibrin sealant as hemostatic or sealing agent
Factor V	Factor V deficiency
Factor VII	Factor VII deficiency
Factor VIII	Hemophilia A
Factor IX	Hemophilia B
Factor X	Factor X deficiency
Factor XI	Hemophilia C (factor XI deficiency)
Factor XIII	Factor XIII deficiency
Von Willebrand factor	Severe forms (type 3 and in type 2) of Von Willebrand factor deficiency, sometimes in combination with factor VIII administration
Factor VIII/Von Willebrand factor	Hemophilia A; severe forms (type 3 and in type 2) of Von Willebrand factor deficiency
Prothrombin complex	Treatment of complex liver diseases; warfarin or coumarin derivatives reversal; hemophilia B (in the absence of single factor IC concentrate)
Fibrin sealant/fibrin glue (fibrinogen and thrombin)	Topical tissue hemostatic healing and sealing agent for surgical applications
Activated prothrombin complex	Hemophilia A with neutralizing anti-FVIII inhibitors (in the absence of other treatment possibly more adapted to patient condition)
Antithrombin	Congenital (or acquired) deficiency leading to thrombosis
Alpha 1-antitrypsin	Congenital deficiency associated with panacinar lung emphysema
C1-esterase inhibitor	Congenital deficiency leading to angioedema
Protein C	Congenital deficiency leading to thrombosis
Polyvalent IgG (normal)	Prevention of infections in immunodeficient patients; Immune modulation in various immunological disorders
Hyperimmune IgG: hepatitis B, hepatitis A, tetanus, rabies, varicella/zoster	Prevention or treatment of infections
Anti-Rho (D)	Prevention of haemolytic disease of the newborn
Albumin	Volume replacement

Multiple plasma-derived products for people with bleeding disorders are in the clinic today.

Not all commercially available (FV concentrate).

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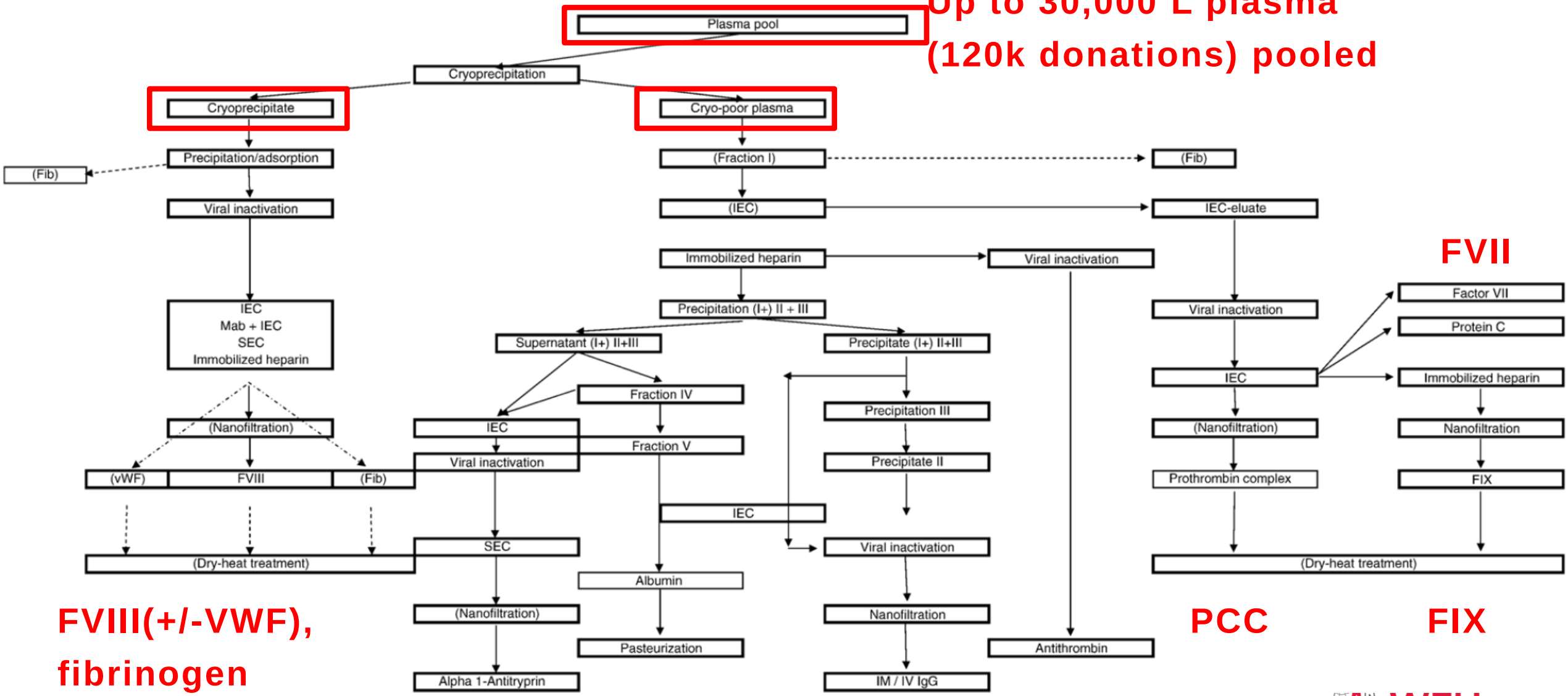
Burnouf T, Ann Blood, 3, 33, 2018

Plasma fractionation today (3)

Up to 30,000 L plasma (120k donations) pooled



FVII

FVIII(+/-VWF), fibrinogen

PCC

FIX

Fib	Fibrinogen
IEC	Ion exchange chromatography
Mab	Monoclonal antibody chromatography
SEC	Size exclusion chromatography
()	indicates optional treatment

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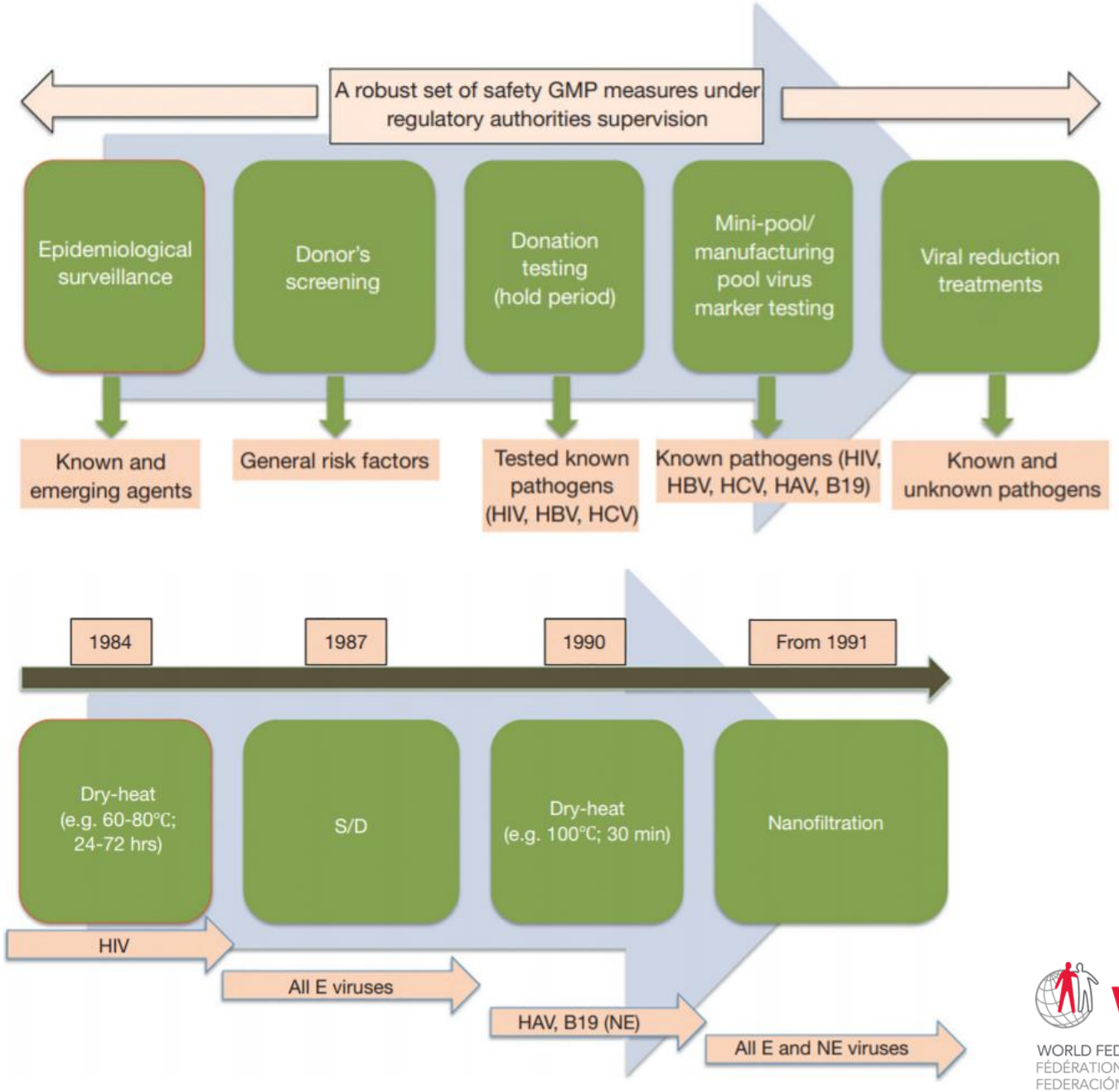
Burnouf T, Trans Med Rev, 21(2), 101-117, 2007

Viral inactivation (1)

The HIV epidemic among PWBDs caused by viral contamination of CFCs prompted integration of viral reduction (inactivation and/or removal) measures in the manufacture of products.



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Burnouf T, Ann Blood, 3, 33, 2018

Viral inactivation (2)

Table 3. Viral Reduction Treatments of Licensed Plasma Products

Treatment	Products	Target viruses in-process treatment	Comments
In-process treatment			
Solvent-detergent	Coagulation factors (eg, FVIII, prothrombin complex, FIX, VWF, fibrinogen)	E	No, or limited, protein denaturation The SD agents are removed by subsequent protein purification steps
	AT		
	IgG		
	Fibrin sealants		
Pasteurization	Coagulation factors (eg, FVIII, fibrinogen)	E Most NE	Protein stabilizers may limit viral inactivation B19 is heat resistant 10% to 30% loss of functional activity of coagulation factors
	IgG		
	AAT		
	AT		
Vapor heat	Coagulation factors (eg, FVIII)	E Most NE	As pasteurization
	C1-inh		
	Fibrin sealants		
Low pH (pH 4) treatment	IgG	E	Most other plasma proteins loose functional activity at low pH
Caprylic acid treatment (<pH 5.5)	IgG and IgM	pH 4 sensitive NE	Most other plasma proteins loose functional activity at low pH
		E	
Nanofiltration	Coagulation factors (eg, FIX, FXI, FVIII, VWF)	E NE	Viral removal by size-exclusion mechanism depends upon virus size and shape, and nanofilter porosity
	IgG		
	AAT		
	AT		
	Fibrin sealants		
Terminal treatment			
Pasteurization	Albumin	E NE	Only for a product withstanding liquid heat-treatment in the presence of small amount of stabilizers
Dry heat	Coagulation factors (eg, FVIII, FIX, prothrombin complex, FXI)	Some E	Inactivation of heat-resistant viruses depends on temperature and duration
		Some NE	Hardly inactivates B19 10% to 20% loss of functional activity of coagulation factors

E indicates enveloped; NE, nonenveloped.

No HIV, HCV or HBV infections via CFCs have been reported since the introduction of the viral reduction measures.

Unknown risk of new infectious agents.

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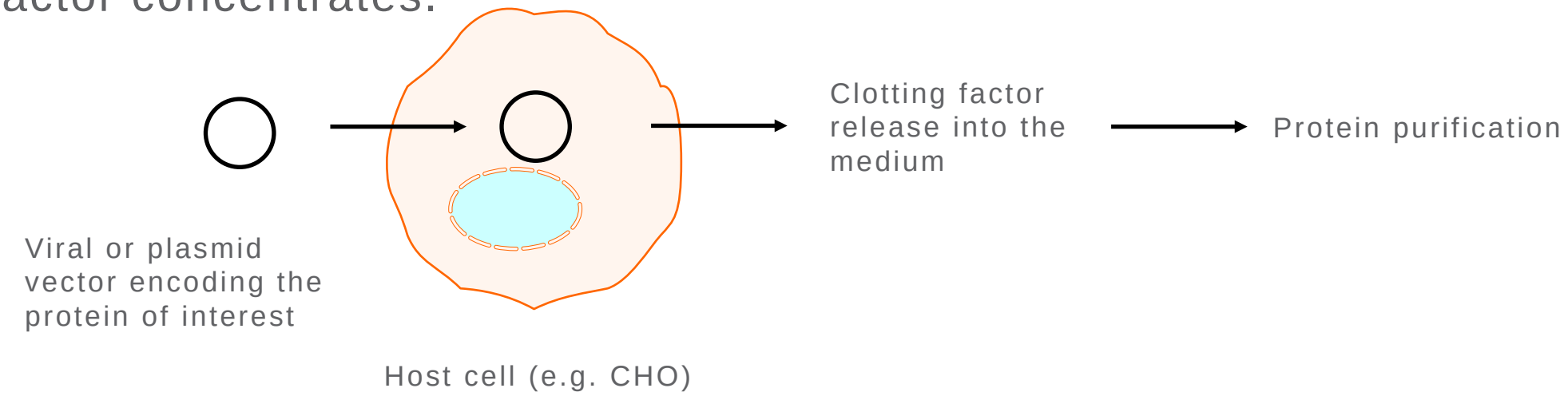
Burnouf T, Trans Med Rev, 21(2), 101-117, 2007

Recombinant factor concentrates (1)

F9 was cloned in 1982 (Choo et al, Nature, 1982).

F8 gene was cloned in 1984 (Vehar et al, Nature, 1984; Wood et al, Nature, 1984; Gitschier et al, Nature, 1984; Toole et al, Nature, 1984).

The need for safer treatment products drove efforts to make recombinant clotting factor concentrates.



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Recombinant factor concentrates (2)

Table 1 Recombinant plasma proteins (licensed or under advanced development)

	Expression system						Regulatory status	
	Mammalian cells			Micro-organisms				
	Animals		Human	Bacteria	Yeast			
Protein	CHO	BHK	HEK	Escherichia coli	Pichia/ Saccharomyces	Transgenic animals	Year licensed in EU (or other countries)	Advanced development
Coagulation factors								
FIX (Benefix)	X						1997	
FIX						X		X
FL-FVIII (Recombinate)	X						1992	
FL-FVIII (Kogenate/Helixate)		X					2000	
FL-FVIII plasma/albumin free (Advate)	X						2004	
BDD-FVIII (ReFacto)	X						1999	
BDD-FVIII plasma/albumin free (Xyntha)	X						2008 (USA)	
BDD-FVIII (Greengene)	X						2009 (Korea)	
BDD-FVIII			X					X [136]
FVIIa (Novoseven)		X					1996	
FVIIa						X		X
Thrombin	X						2008[a] (USA)	
VWF	X [64]						–	X
VWF						X [66]	–	X
Fibrinogen						X [26,73]	–	X
FXIII					X		–	X

Three mammalian cell lines mostly used (CHO, BHK, HEK).

rFXIII now available and manufactured in glycoengineered *Pichia pastoris* (yeast).

Recently approved new rFVIIa purified from milk of transgenic rabbits.

rVWF now available.

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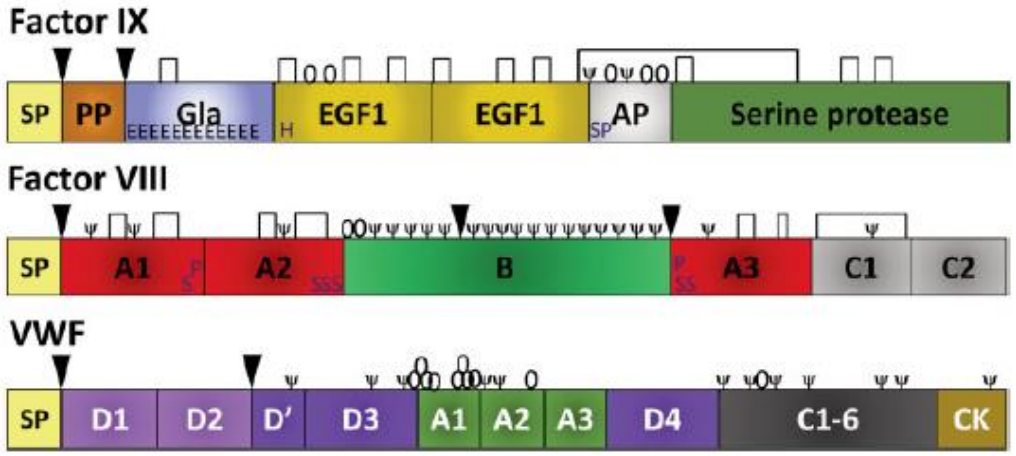
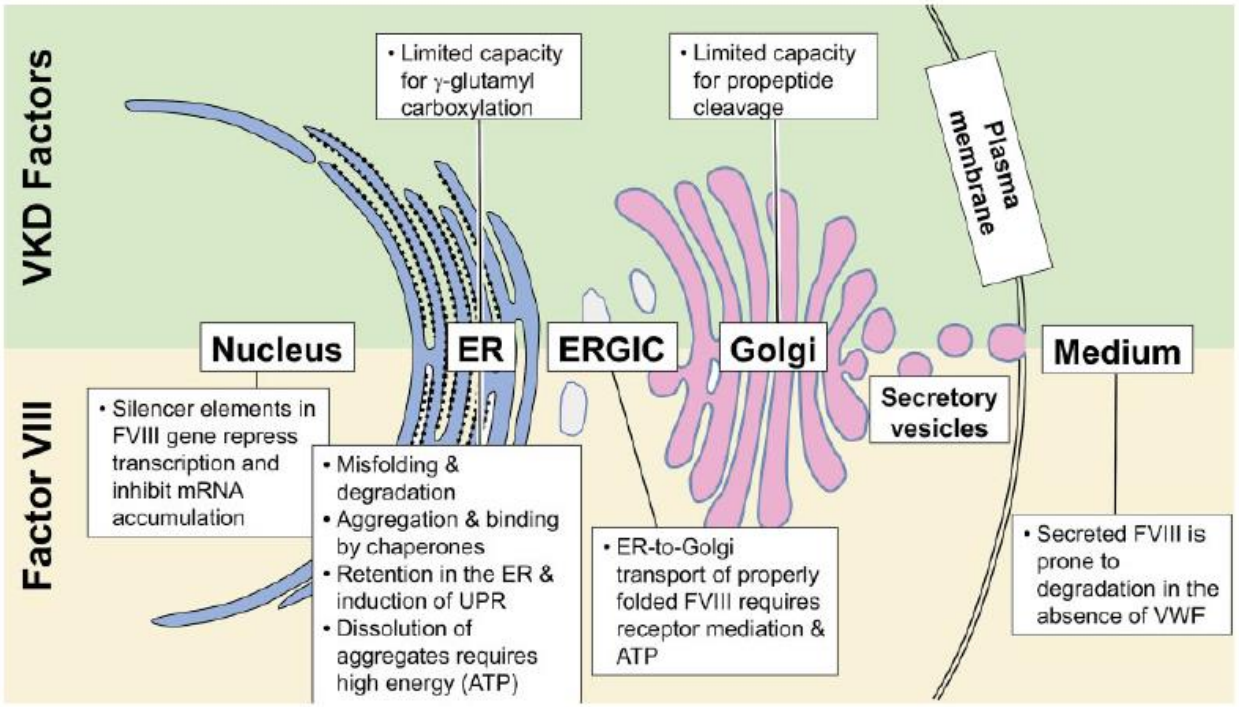
Burnouf T, Vox Sang, 100, 68-83, 2010

Recombinant factor concentrates (3)





Multiple complex posttranslational modifications limit the choice of workhorse cell lines.

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Kumar SR, Biotechnol J, 10, 995-1004, 2015

Extended half-life factors

Table 1
Extended half-life products available and/or in development

Product	Ref	Technology	Half-Life (h)	Cell Line	FDA Approval
FVIII					
rFVIII-Fc (Eloctate/Elocta)	44–47	Fusion protein of BDD rFVIII and the Fc fragment of IgG1	19	HEK	Jun 2014
BAX 855 (Adynovate/ Adynovi)	48–50	Random PEGylation to parent drug Advate (full length rFVIII)	14–16	CHO	Dec 2016
BAY94–9027 (Jivi)	51–54	Site-specific addition of PEG side chain to a BDD rFVIII	19	CHO	Aug 2018
N8-GP	55,56	Site-specific glycoPEGylation of BD-modified FVIII	19	CHO	NA
BIVV001	57	Fusion protein with addition of a region of VWF and XTEN polypeptides	37	HEK	NA
FIX					
rFIX-Fc (Alprolix)	11,58,59	Fusion protein with the Fc fragment of IgG1	82	HEK	Mar 2014
rFIX-FP (Idelvion)	10,60	Fusion protein with recombinant albumin	102	CHO	Mar 2016
N9-GP (Rebinyn/ Refixia)	9,61	Site-specific glycoPEGylation	93	CHO	May 2017

Several EHL products approved since 2014.

Half-life extension achieved via PEGylation or fusion with Fc fragment of IgG or with albumin.

New technologies under development (e.g. FVIII fusion with Fc, VWF D'D3 and XTEN).

Made in CHO or HEK cells.

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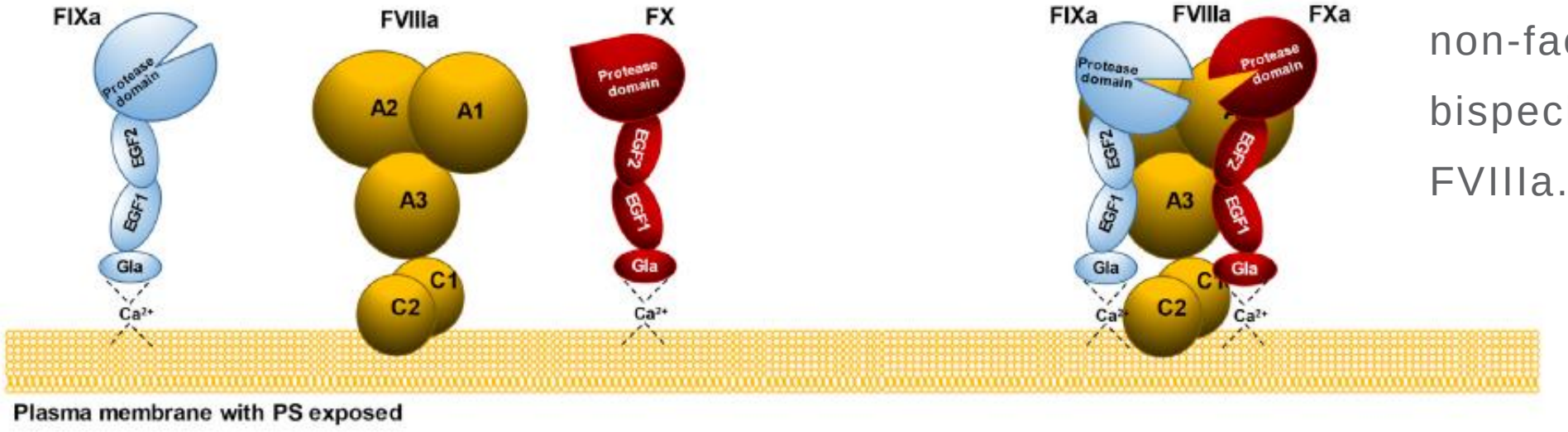
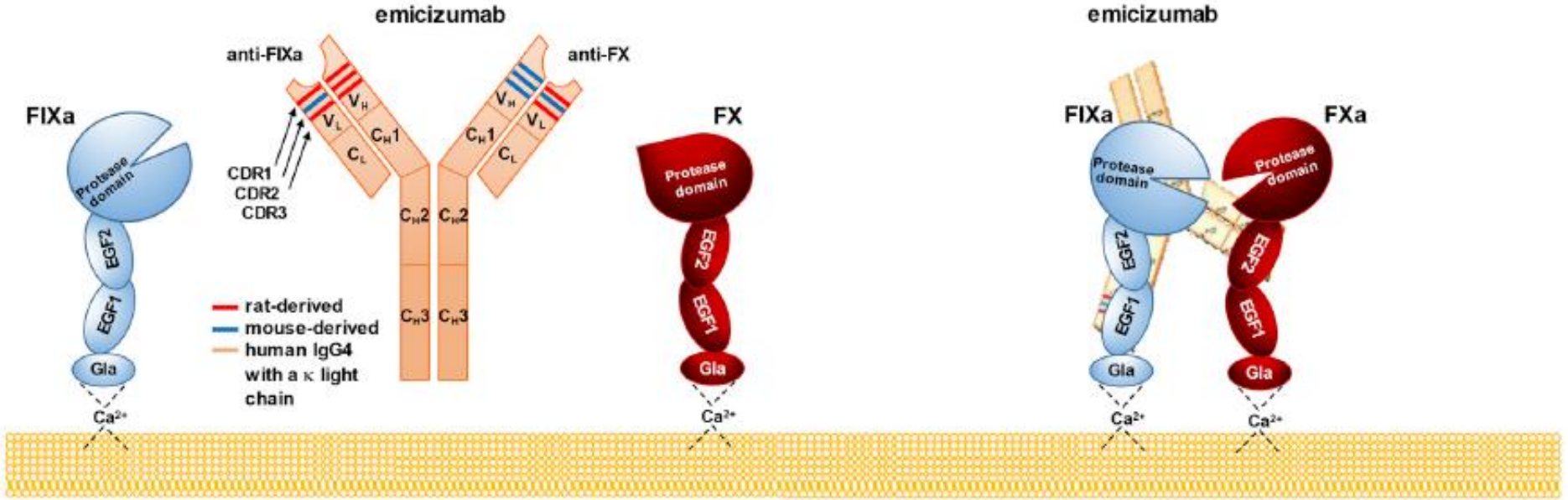
Pelland-Marcotte MC et al, Hematol Oncol Clin N Am, 33, 409–423, 2019

Non-factor therapies (1)

The only approved (2017) non-factor product is a bispecific antibody mimicking FVIIIa.





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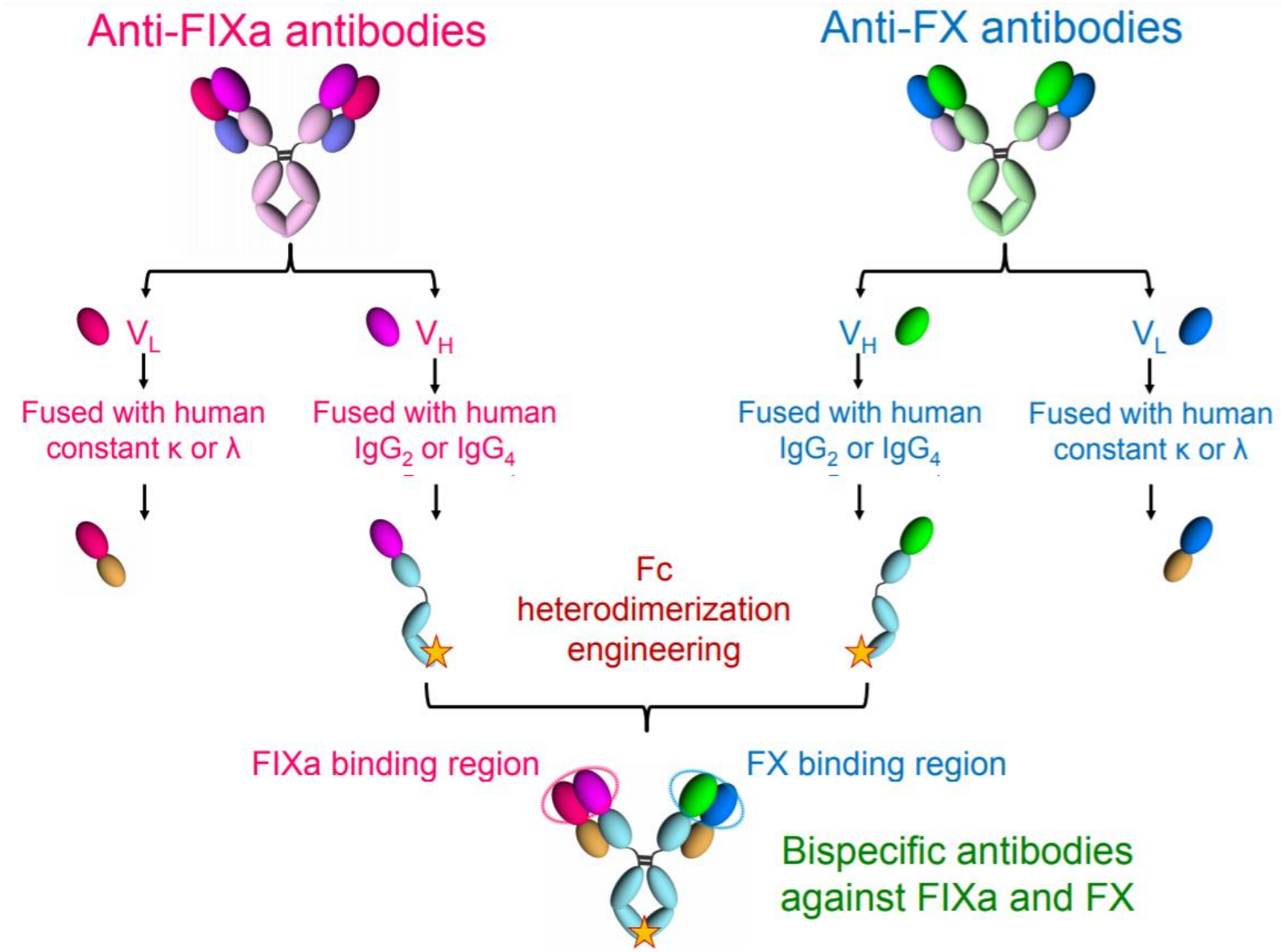
Butterfield JSS, Hege KM, Herzog RW, Kaczmarek R, et al, Mol Ther, 28(3), 2020

Non-factor therapies (2)



Emicizumab was developed via recombination of anti-FIXa and anti-FX antibodies with optimal binding properties.

Made in CHO cells.

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Sampei Z et al, PLoS ONE, 8(2), e57479, 2013

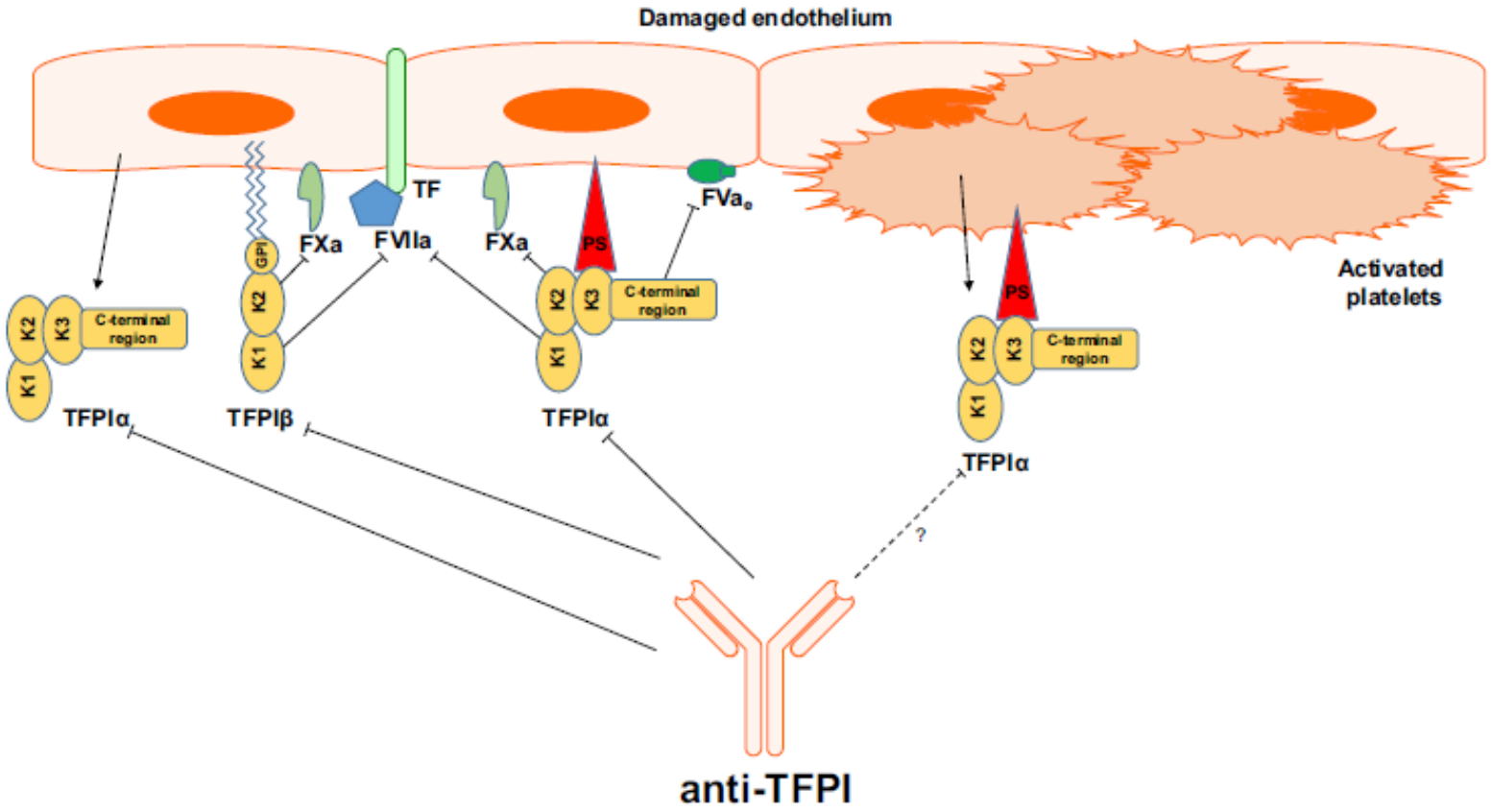
Non-factor therapies (3)

Three anti-TFPI antibodies under development (in CHO):

concizumab (murine mAb, humanized, IgG4)

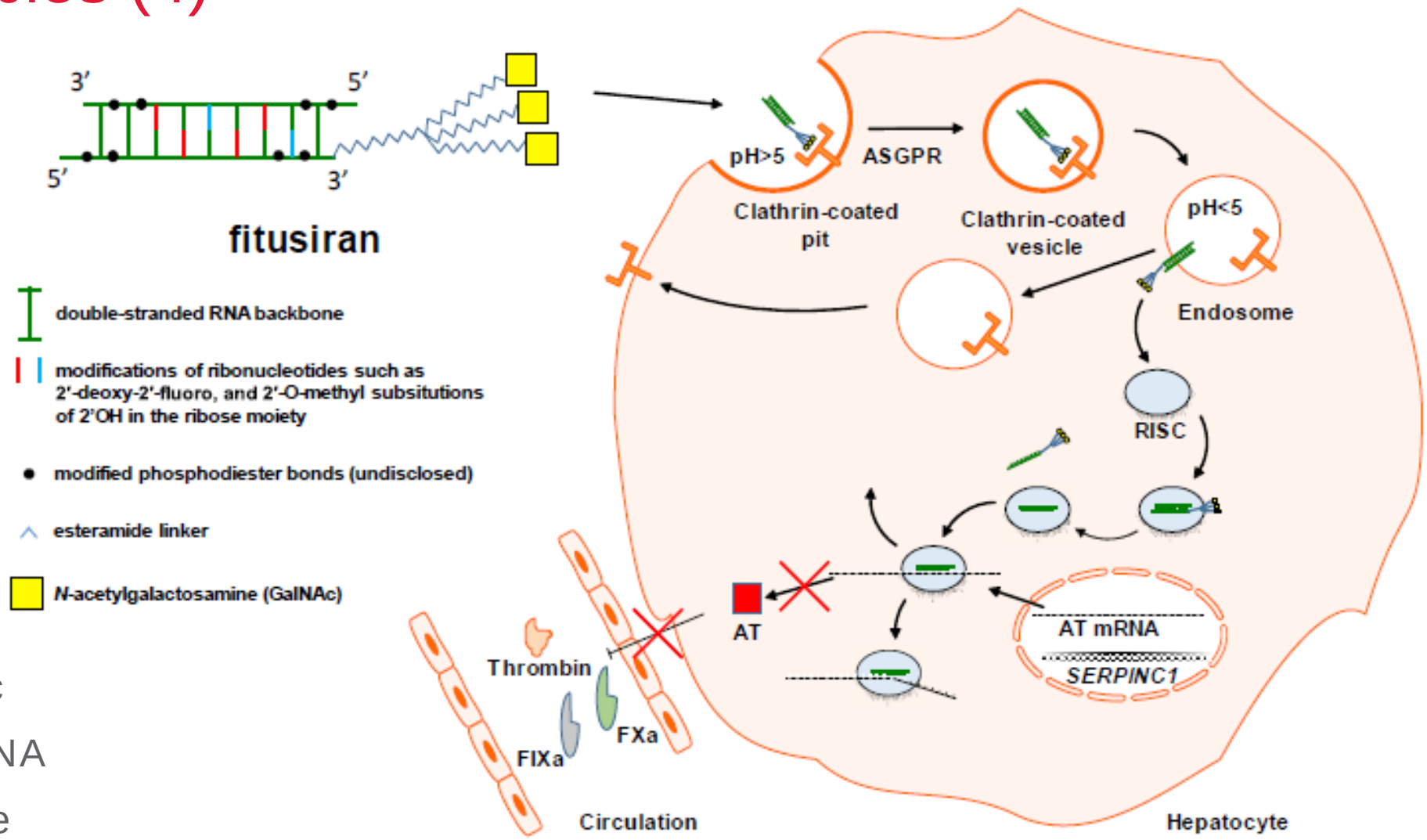
marstacimab (fully human IgG1 mAb, selected from a phage display library of scFvs derived from non-immunized human donors and converted to IgG1)

MG1113 (murine, humanized, IgG4)



Butterfield JSS, Hege KM, Herzog RW, Kaczmarek R, et al, Mol Ther, 28(3), 2020

Non-factor therapies (4)



Fitusiran is a synthetic small-interfering (si)RNA which knocks down the antithrombin transcript.

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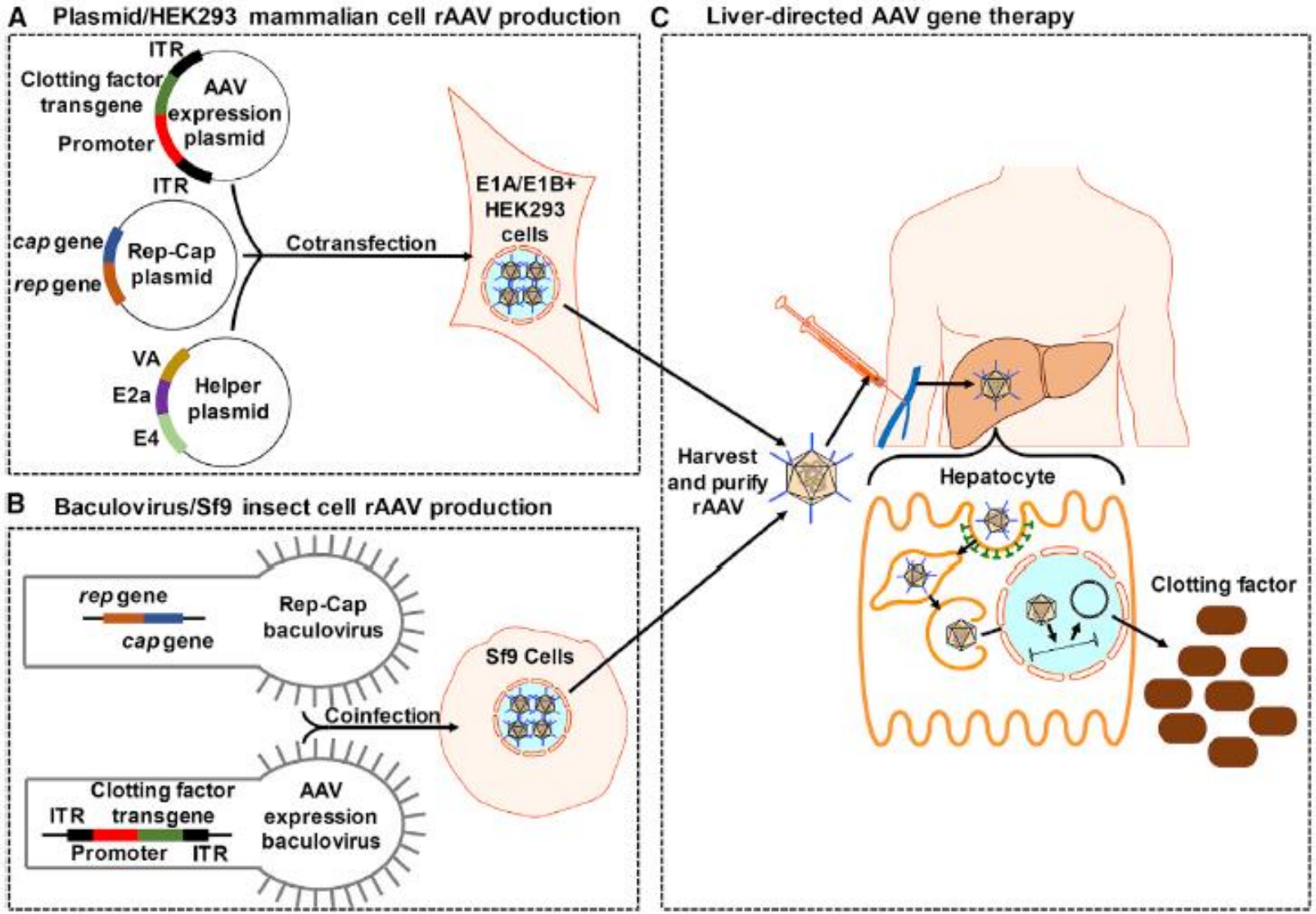
Butterfield JSS, Hege KM, Herzog RW, Kaczmarek R, et al, Mol Ther, 28(3), 2020

Gene therapy

Most AAV vectors tested in clinical trials for hemophilia are made in HEK cells or *Spodoptera frugiperda* 9 (Sf9) cells (insect, lepidopteran).



Butterfield JSS, Hege KM, Herzog RW, Kaczmarek R, et al, Mol Ther, 28(3), 2020

THANK YOU

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Navigating Rebalancing Agents in Clinical Trials

John Pasi, MB ChB PhD

Professor of Haemostasis and Thrombosis

Barts and the London School of Medicine and Dentistry

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Emerging agents for haemophilia

- Next generation coagulation factors
  - EHL + VIII / IX
  - Super IX
- Mimicking clotting factors
  - Bispecific antibody to IX, X
    - 2nd generation bispecific antibody
- Suppressing clot regulators
  - RNAi targeting AT
  - Anti-TFPI
  - APC-Serpin
- Gene therapy
  - AAV Factor IX
  - AAV Factor VIII

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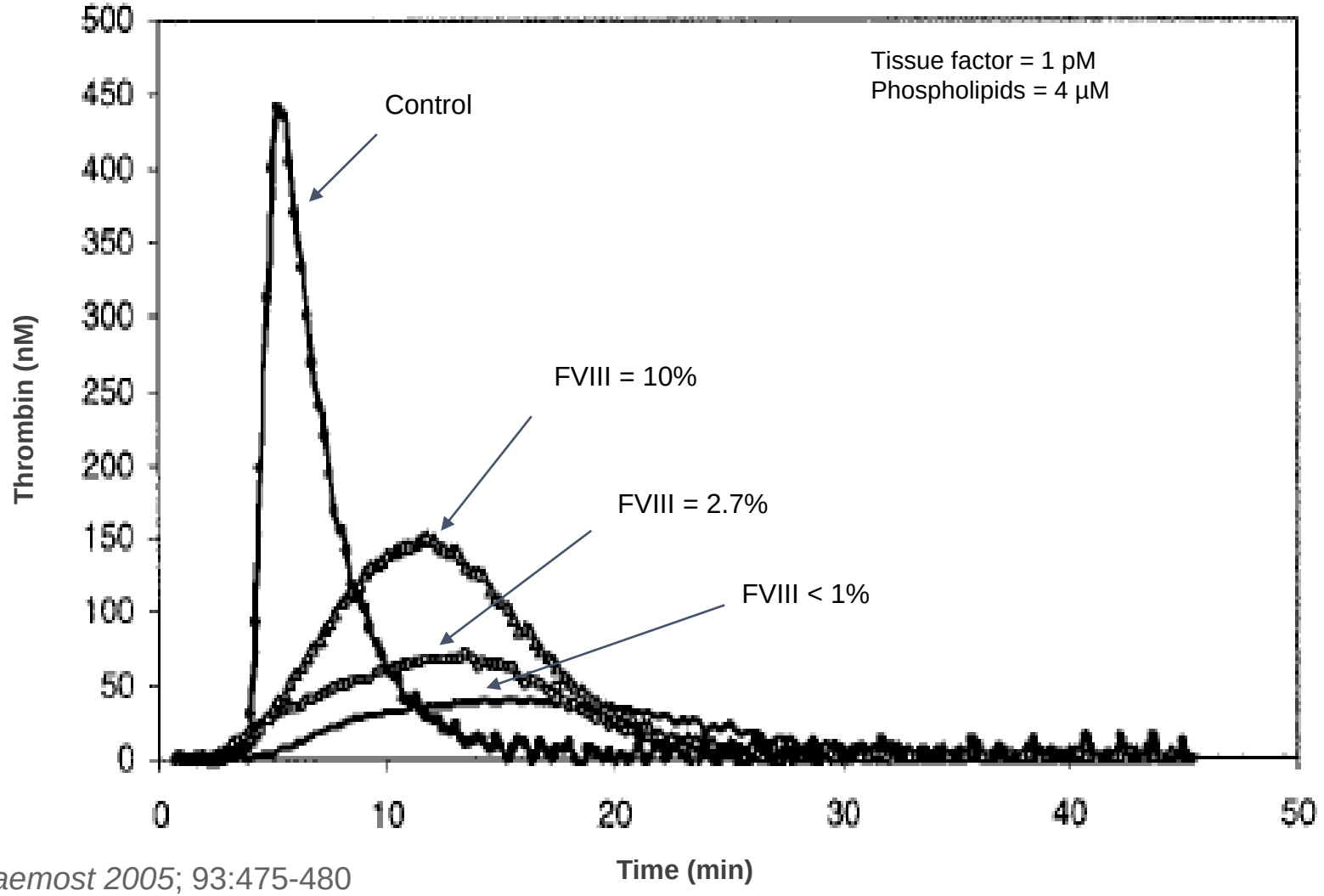
Concepts

- Haemophilia is disorder of thrombin generation
- Natural anticoagulants are major regulators of haemostasis
- Opposing or suppressing natural anticoagulants will rebalance haemostasis in the absence of factor VIII or IX
- Subcutaneous administration

Thrombin Generation and Haemophilia

Thrombin generation correlates with disease severity



Dargaud *et al., Thromb Haemost 2005*; 93:475-480

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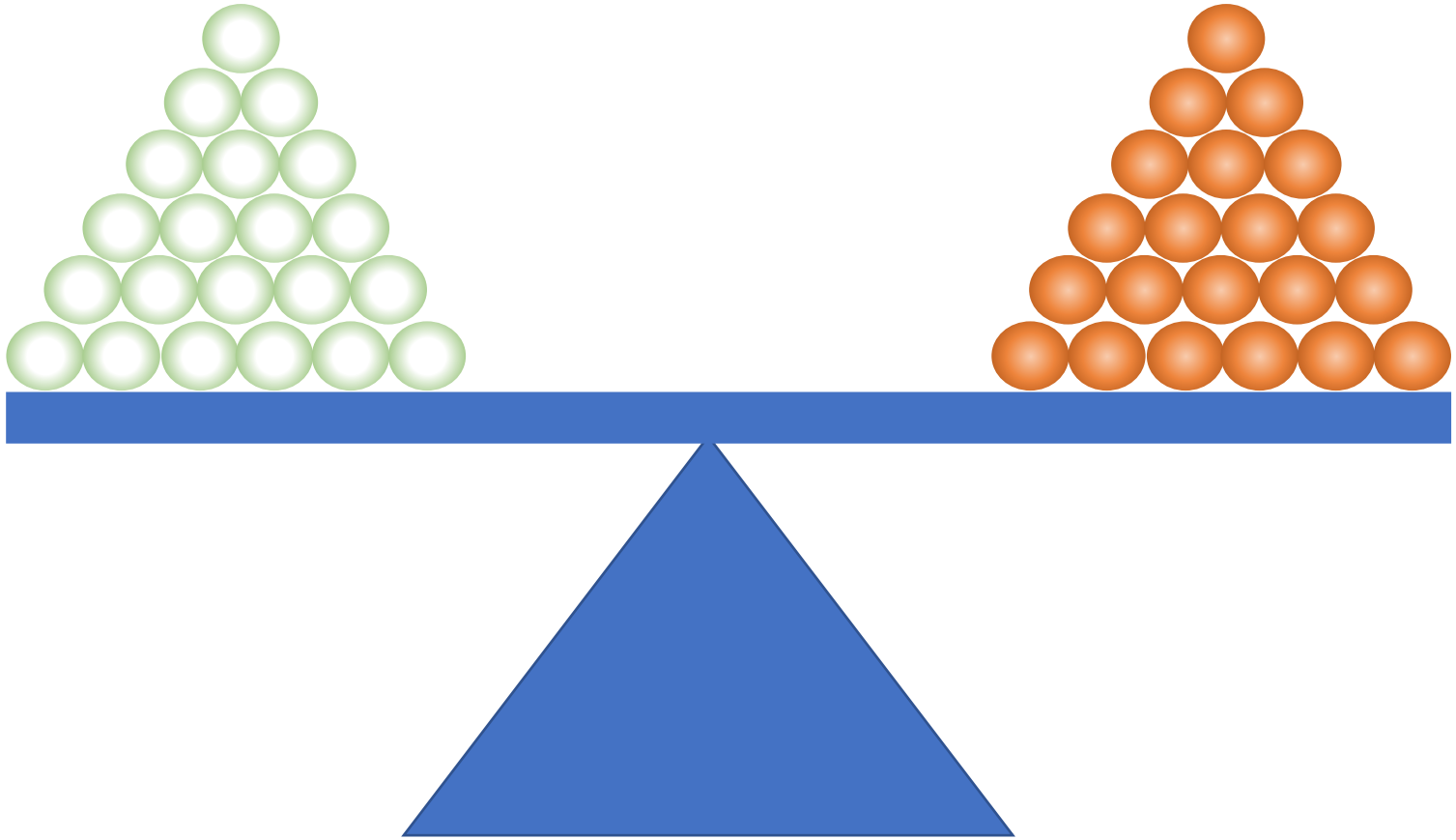
Haemostatic balance, normal



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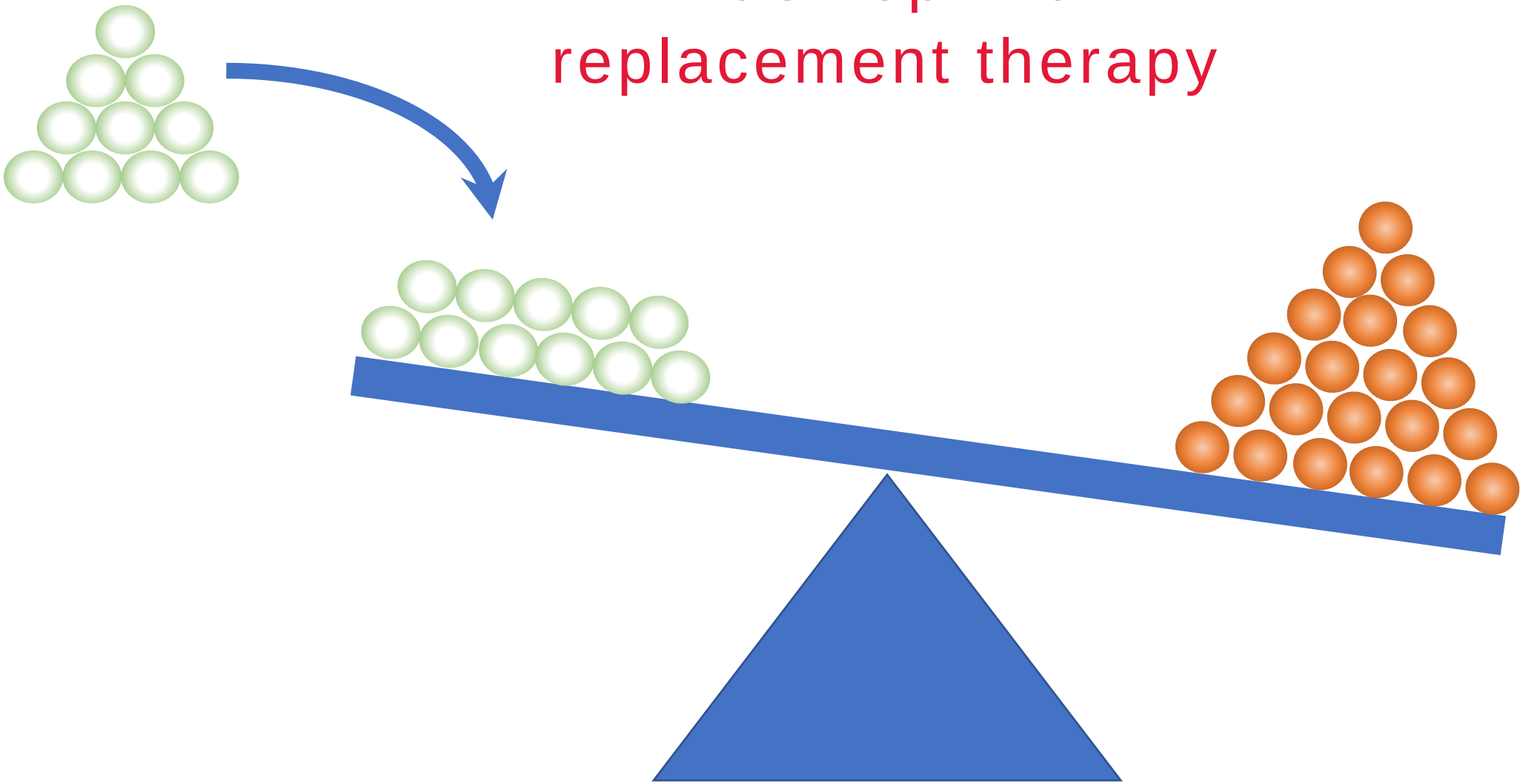
Haemostatic balance haemophilia replacement therapy



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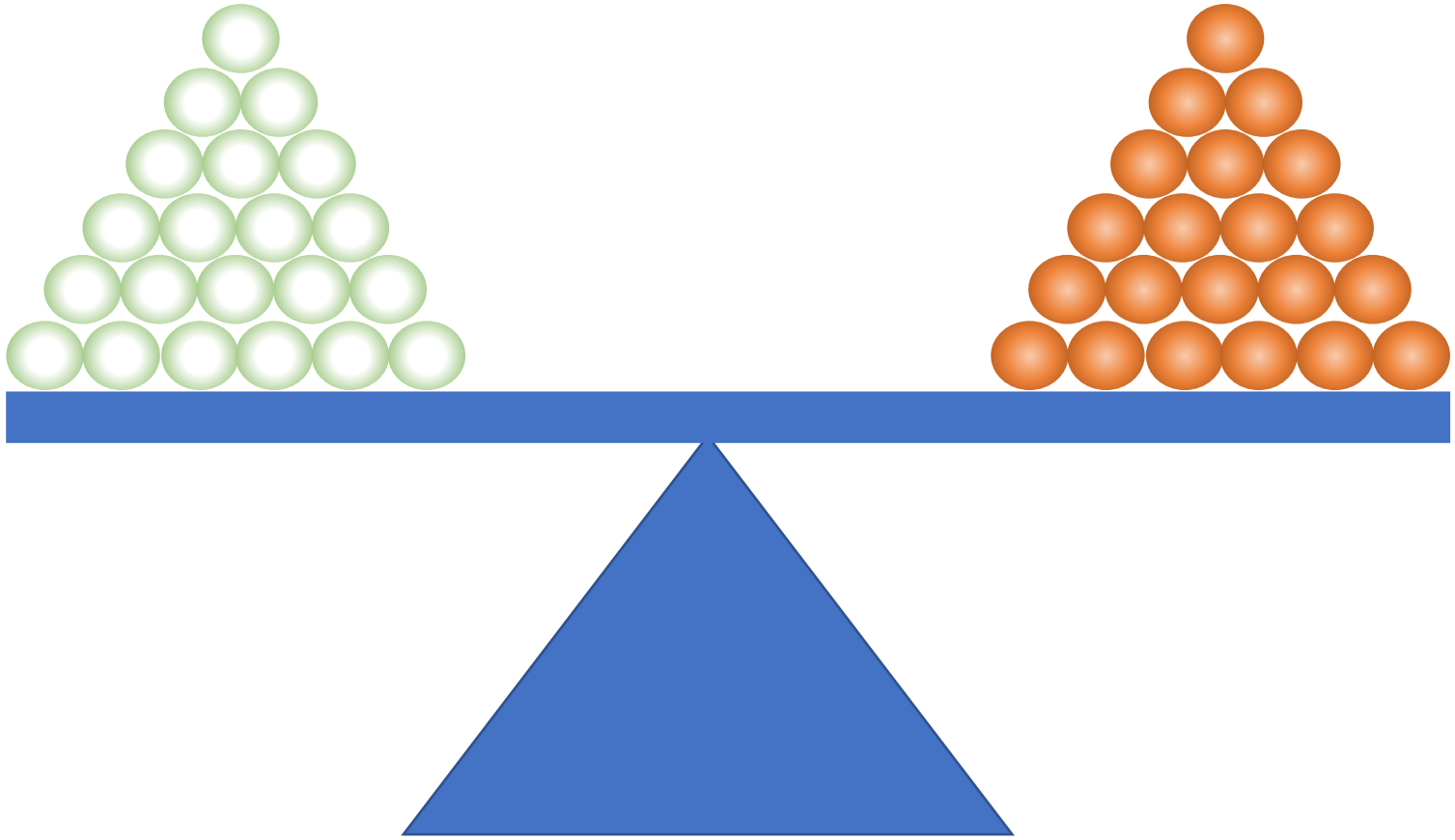
Haemostatic balance



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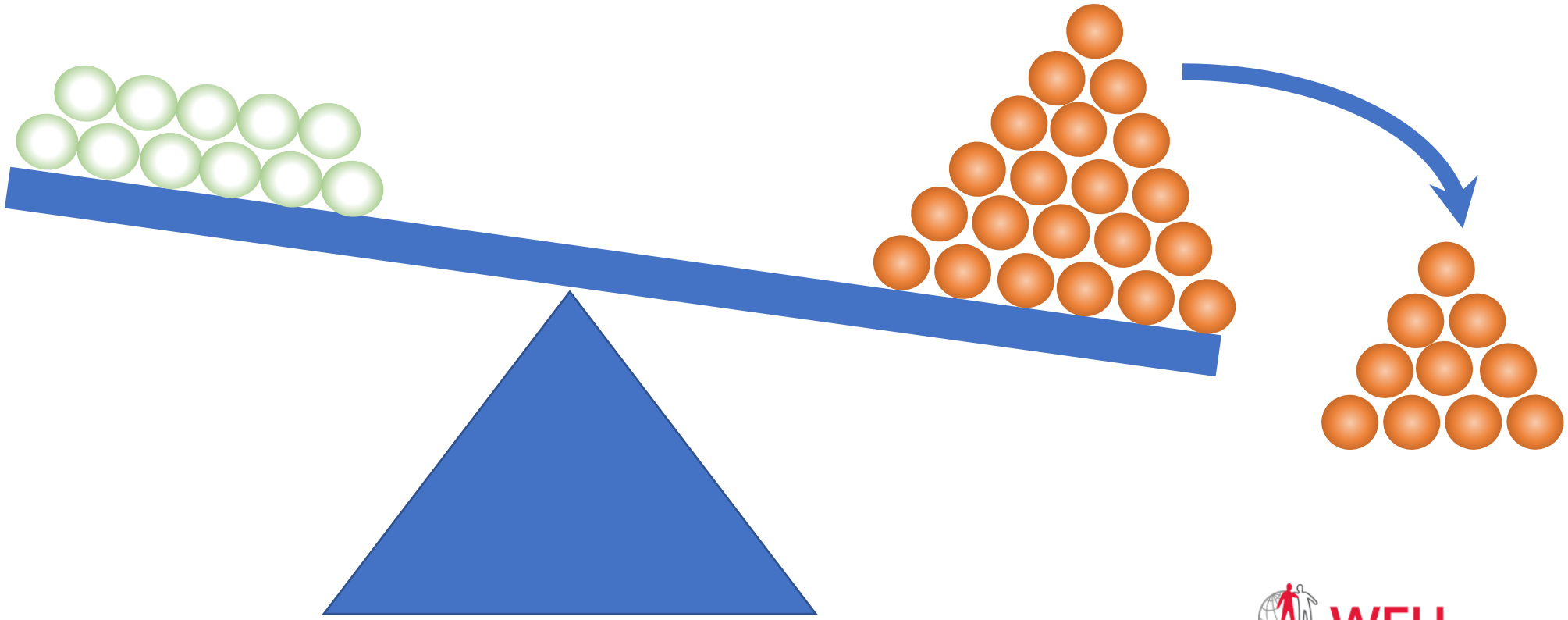
Haemostatic balance
haemophilia
anticoagulant focused



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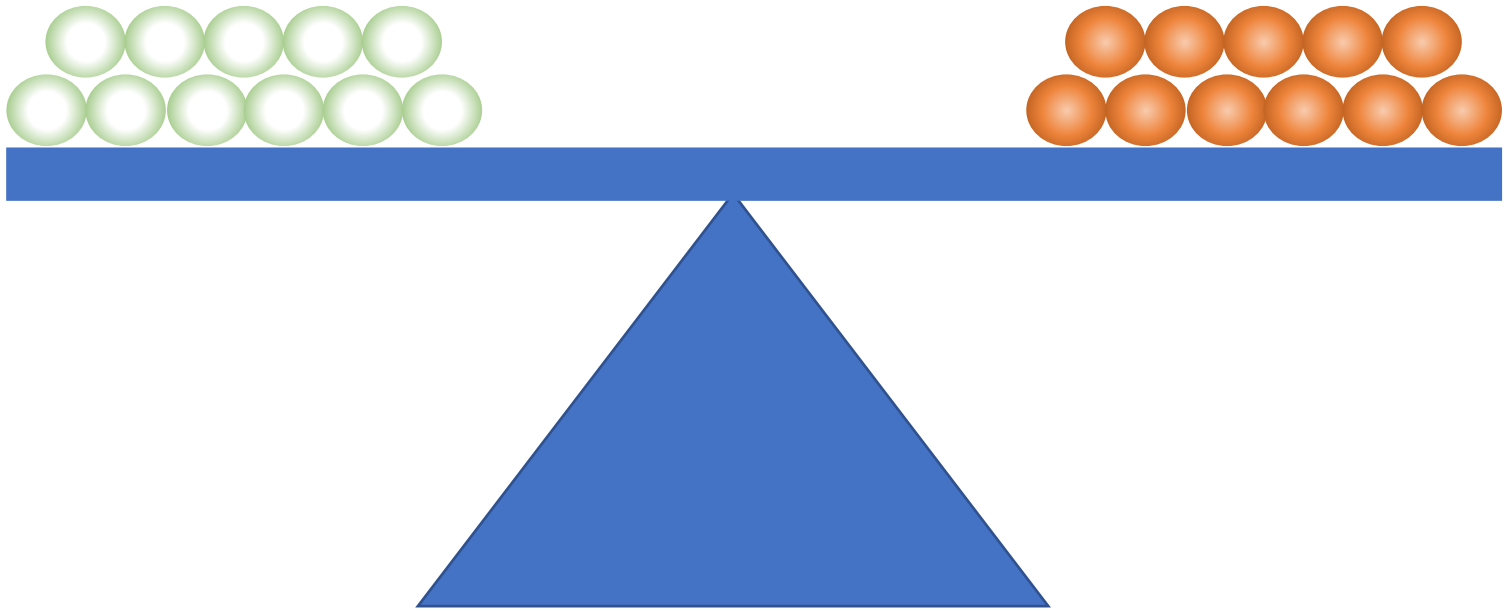
Haemostatic balance



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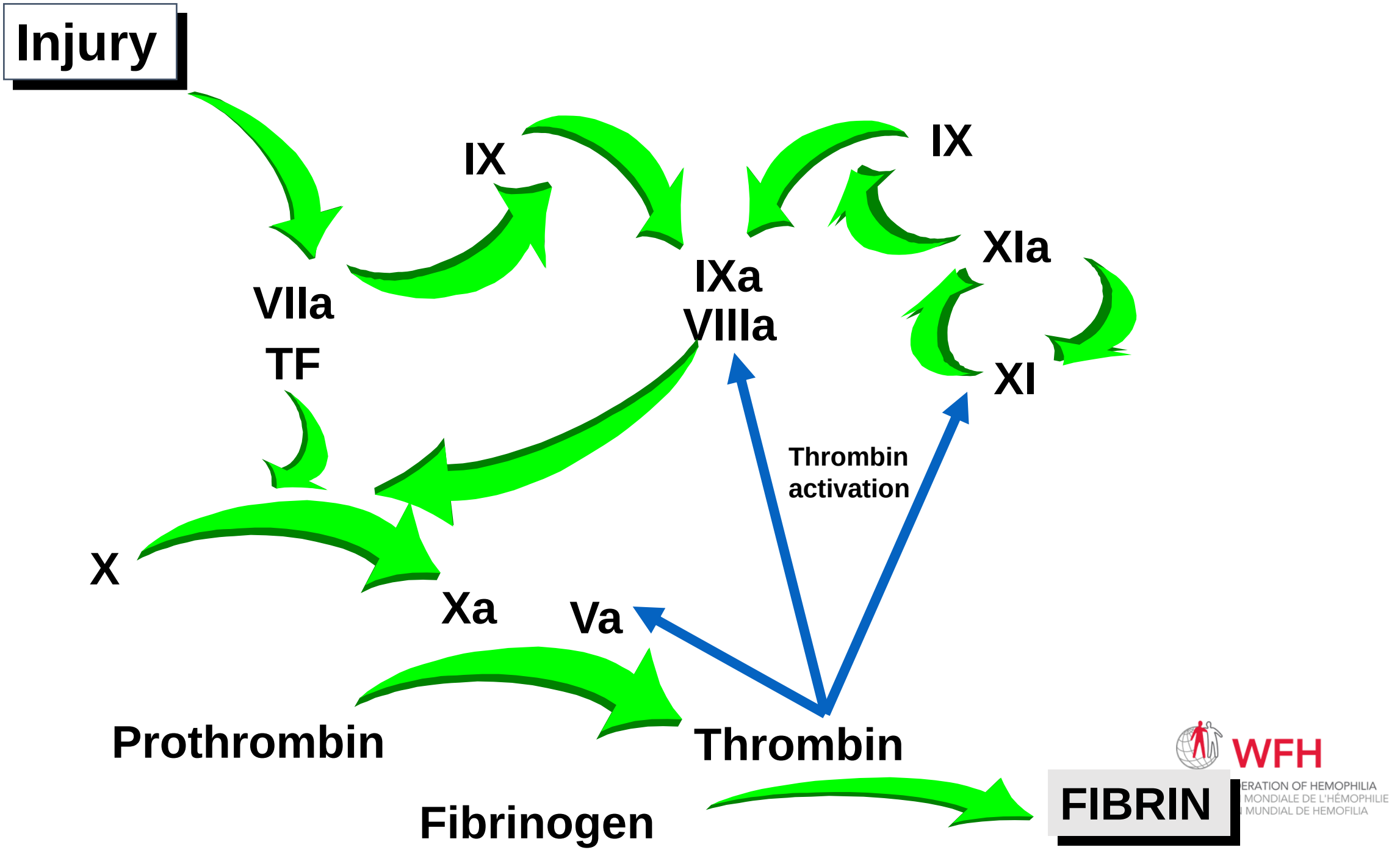
Injury
IX
IX
VIIa
TF
IXa
VIIIa
XIa
XI
Thrombin
activation
X
Xa
Va
Prothrombin
Thrombin
Fibrinogen
FIBRIN
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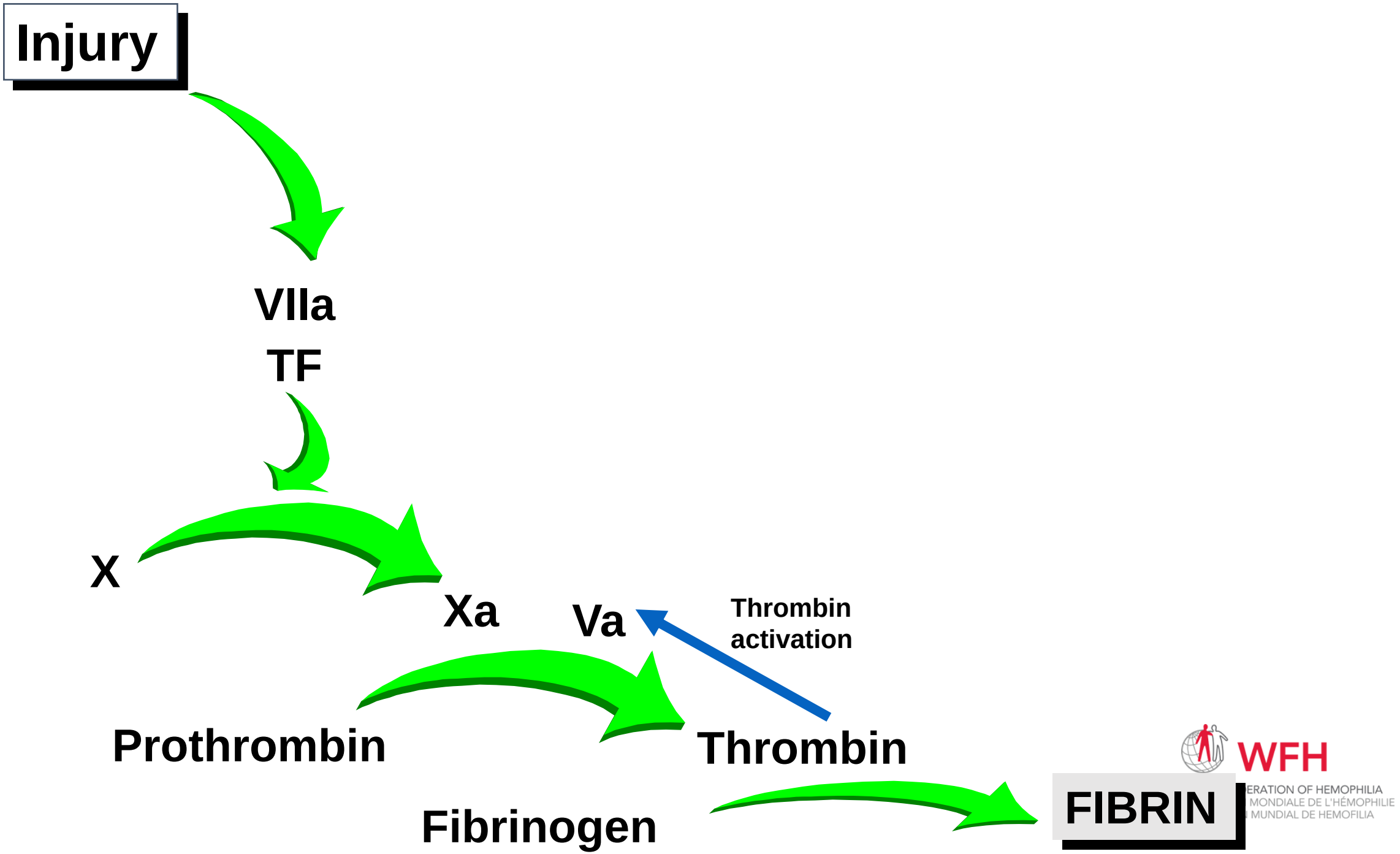
Injury
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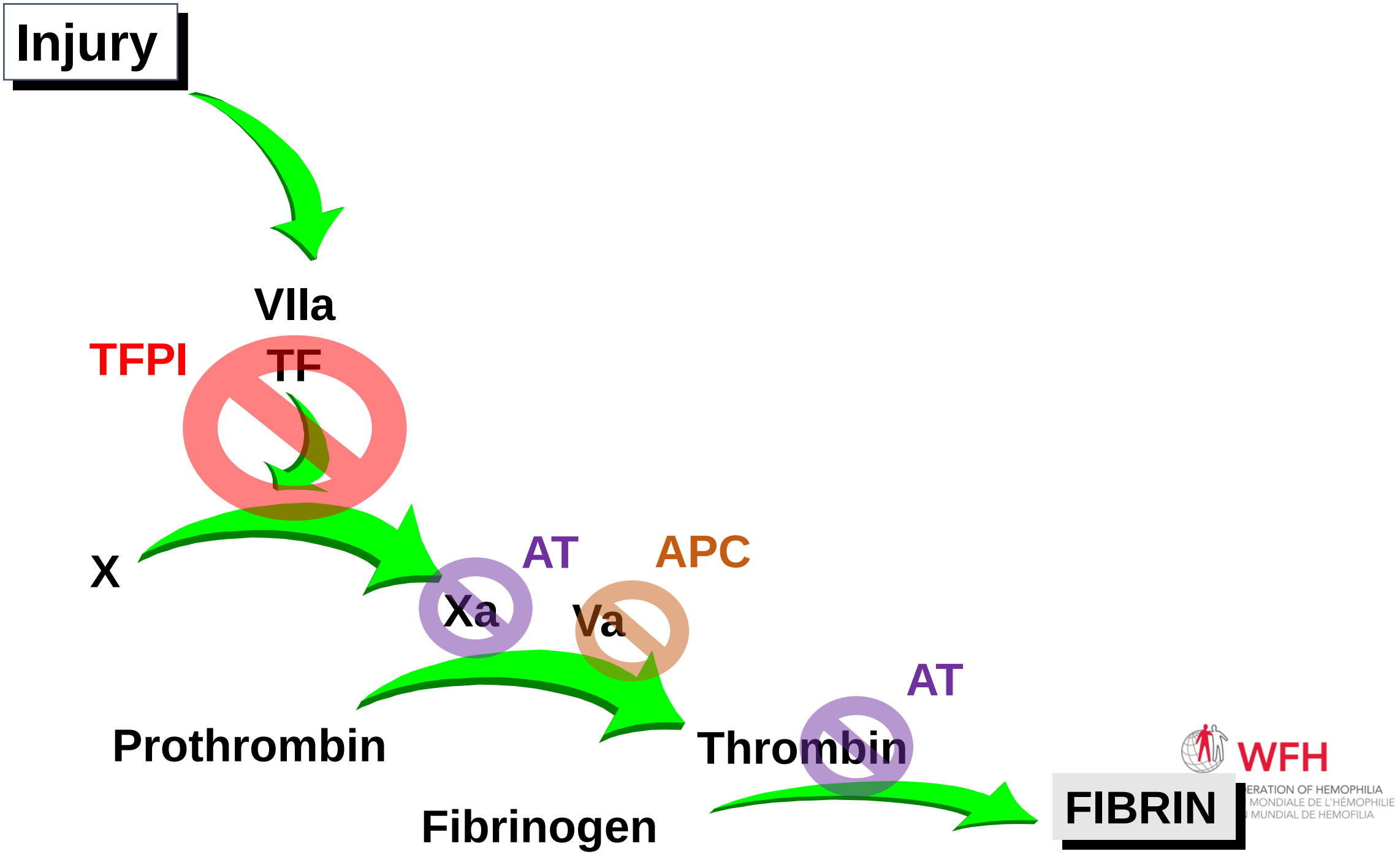
Injury
VIIa
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TF
X
AT
APC
Xa
Va
Prothrombin
AT
Thrombin
Fibrinogen
FIBRIN
WFH
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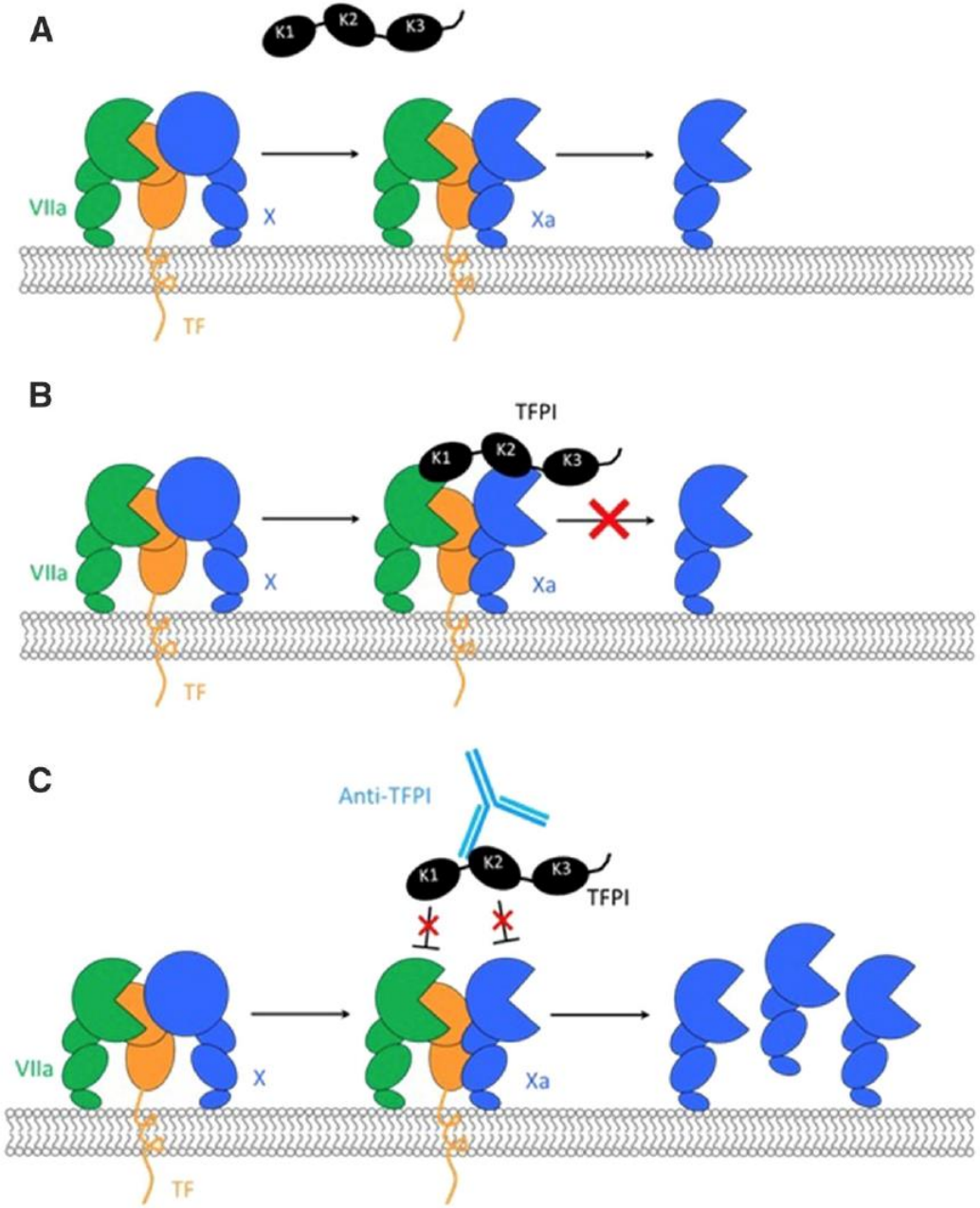
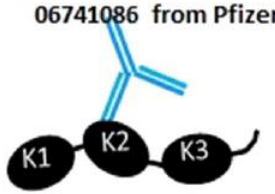
Anti-TFPI



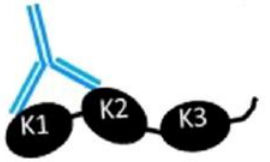
Anti-TFPI antibody against K2 domain

Concizumab and PF-06741086 from Pfizer



Anti-TFPI antibody against K1 & K2 domain

BAY-1093884 from Bayer



Target mediated drug disposition
Daily, biweekly or weekly dosing

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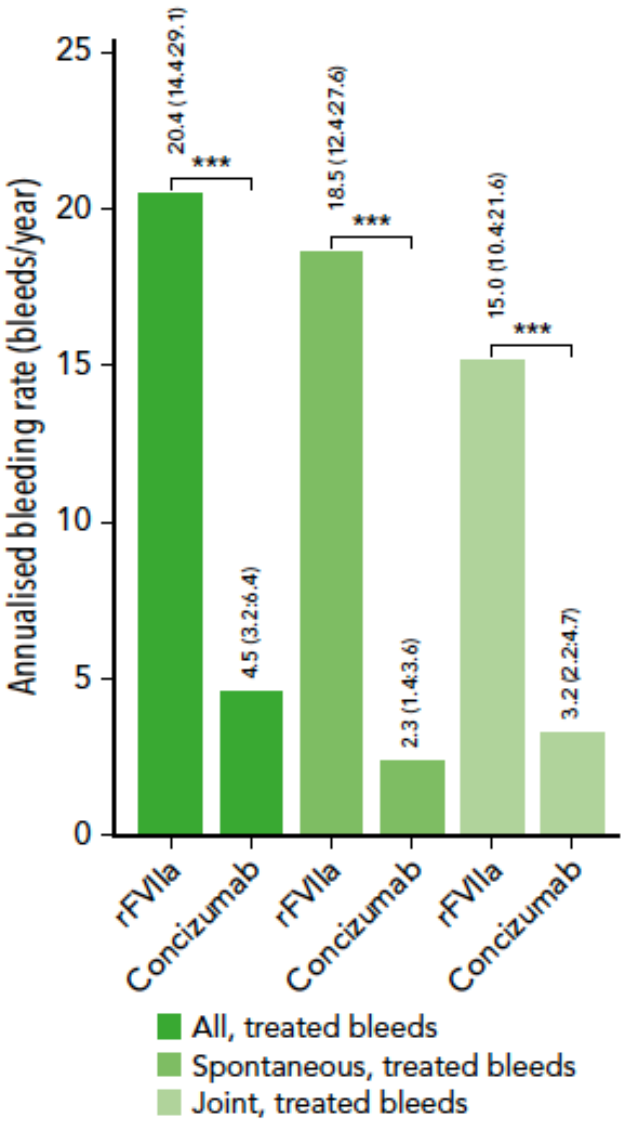
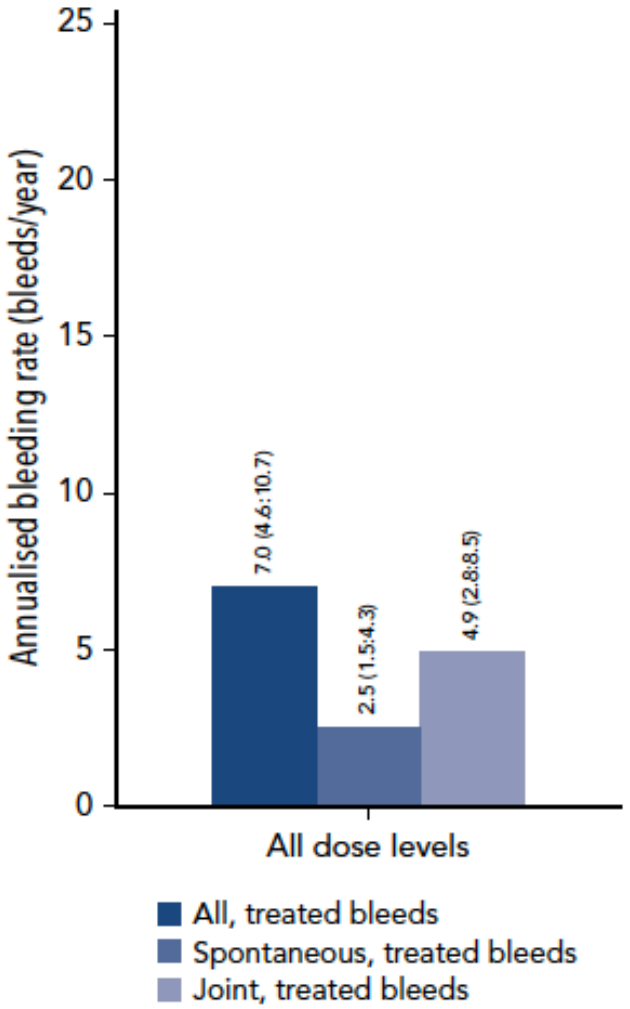
Chowdhury, *Drugs* 2018, 78:881-90

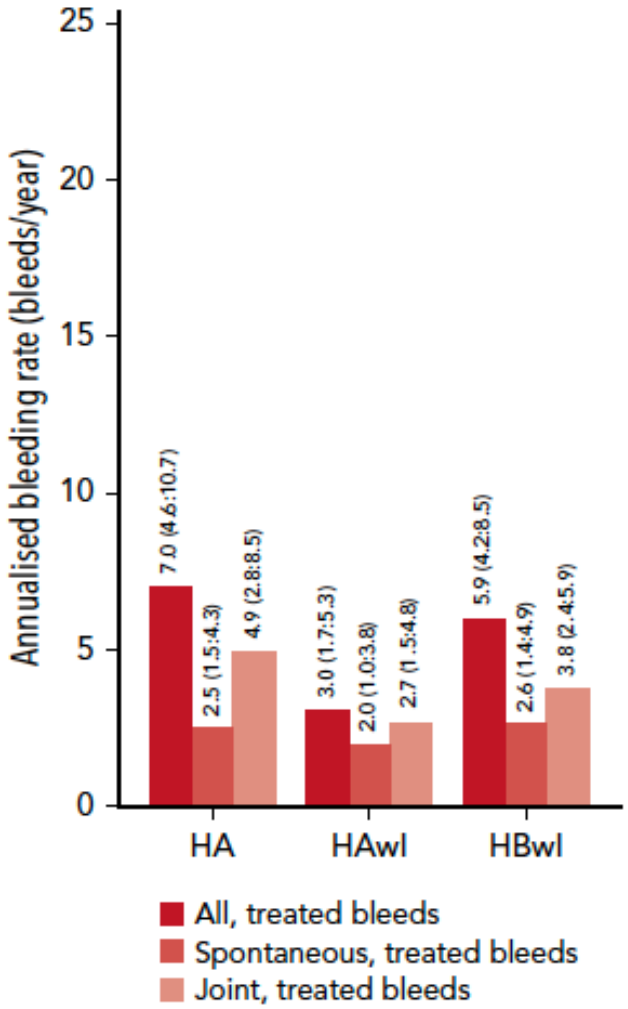
Concizumab phase 2 data



Inhibitor



Non-Inhibitor



By type

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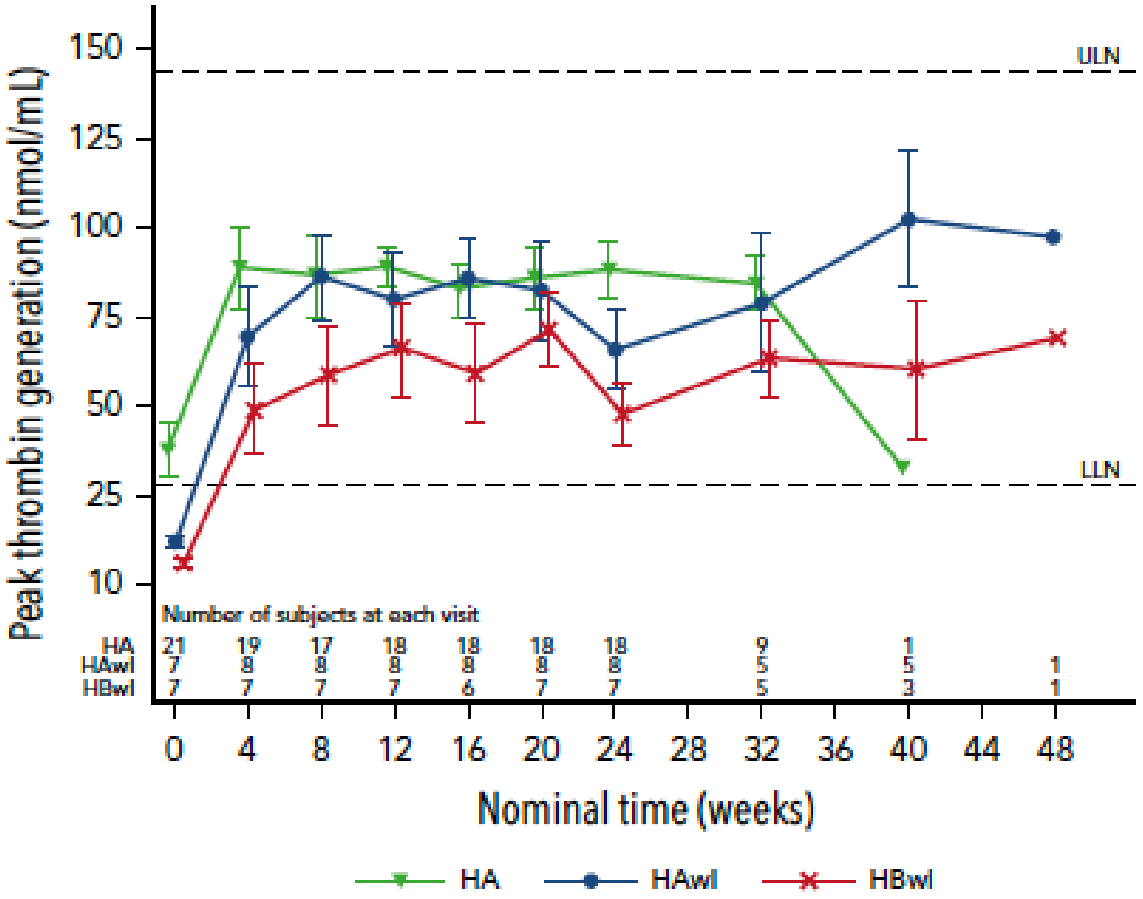
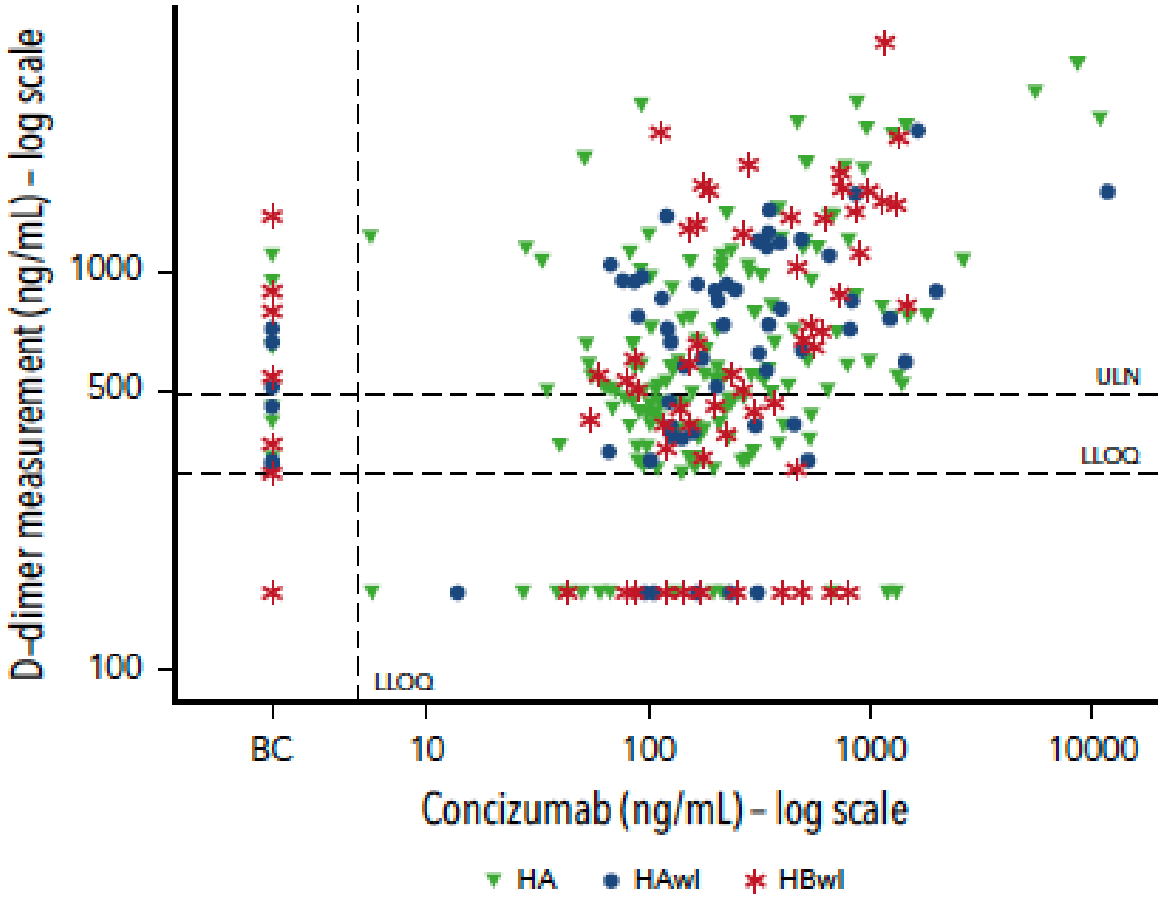
Shapiro et al, *Blood* 2018, 134:1973-82

Concizumab phase 2 data





Shapiro et al, *Blood* 2018, 134:1973-82

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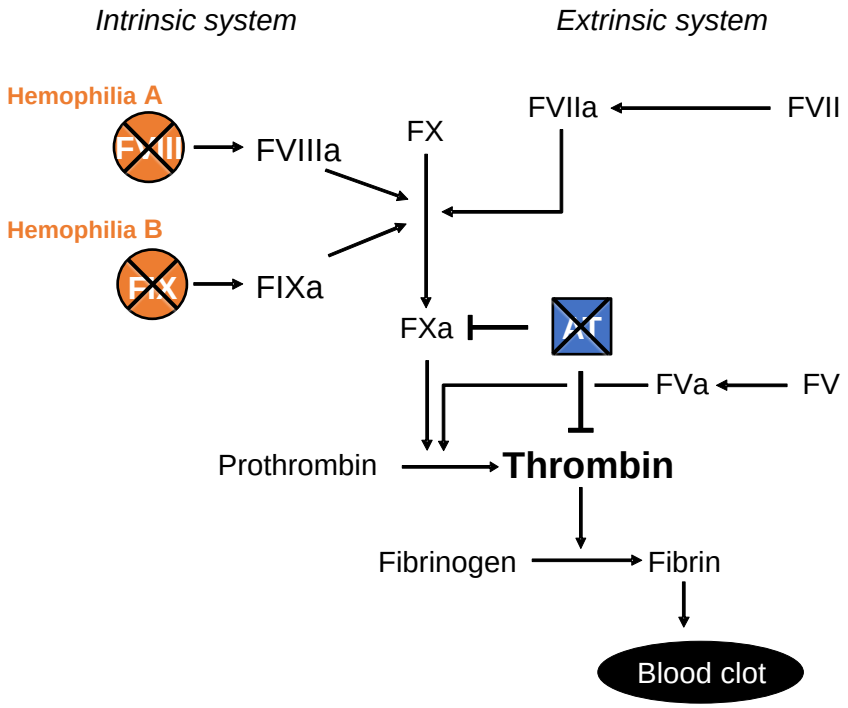
Antithrombin and haemophilia?

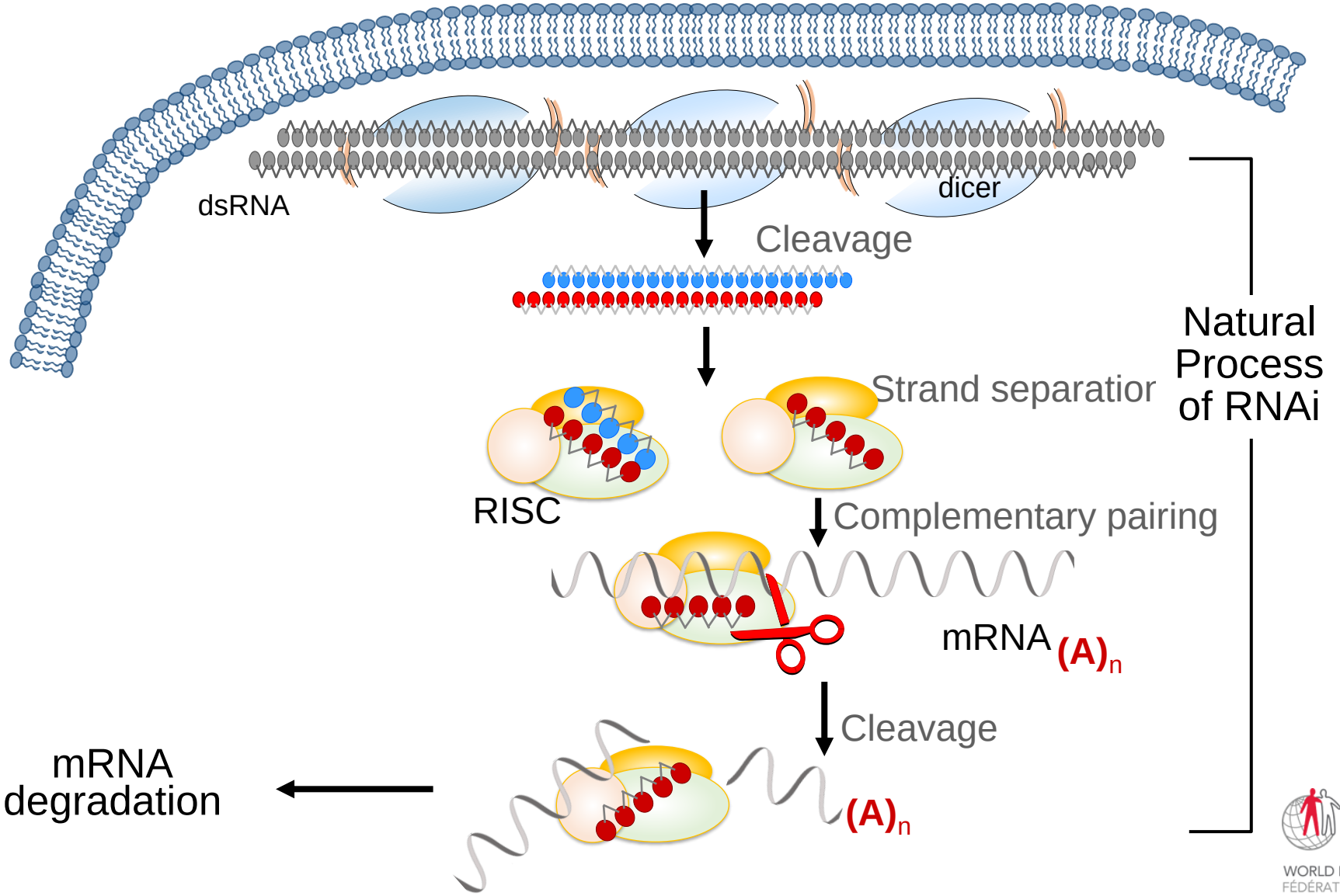
- Antithrombin (AT) as a defined target
  - AT is key natural anticoagulant
    - Inactivates Factor Xa and thrombin
    - Attenuates thrombin generation
  - Human AT deficiency associated with increased thrombin generation
  - Expressed in liver



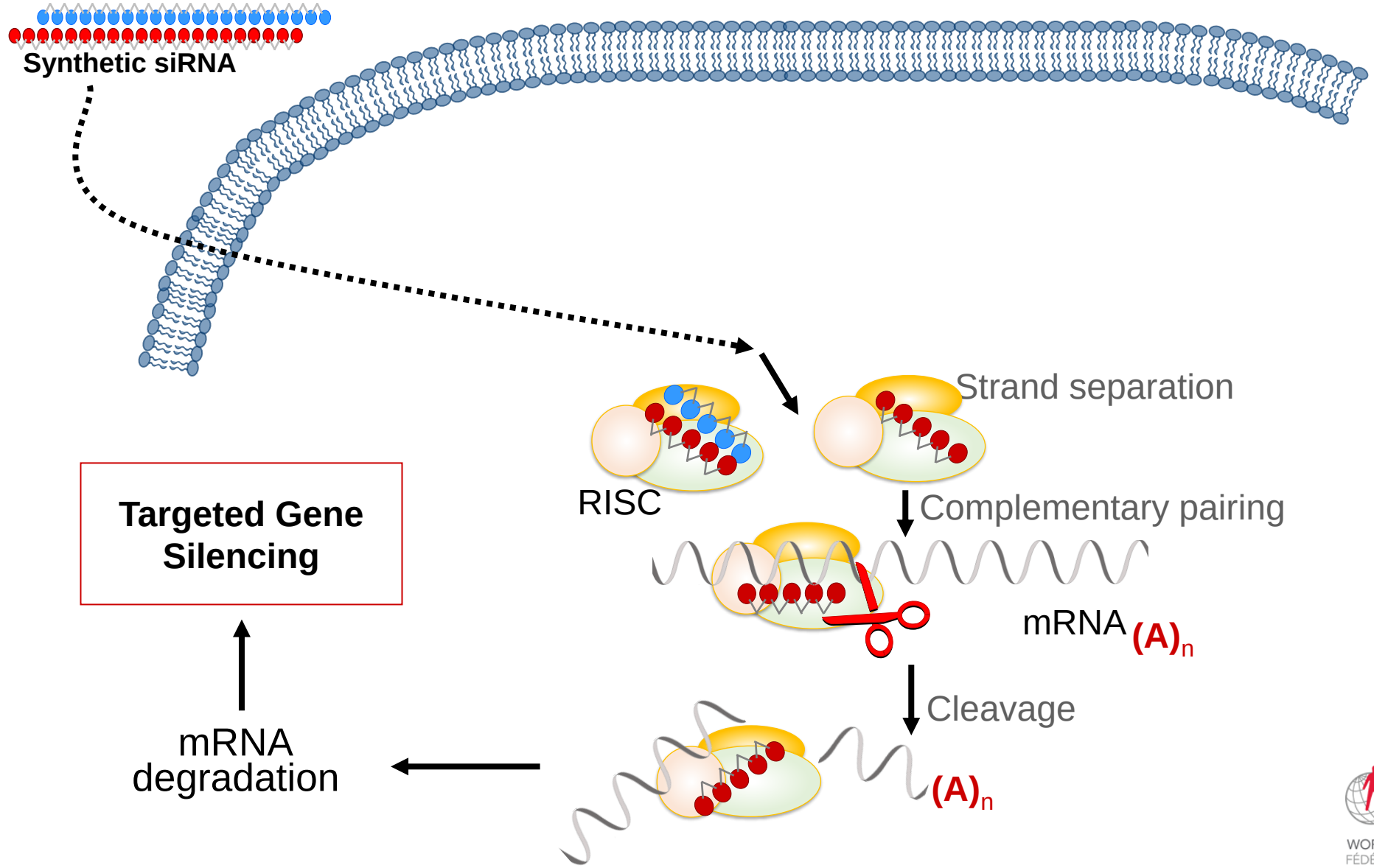
- Co-inheritance of Antithrombin deficiency in haemophilia
  - Associated with milder bleeding, reduced factor requirements, fewer complications

Kurnik *et al., Haematologica* 2007*;* 92:982-5, Ettingshausen *et al., Thromb Haemost* 2001*;* 85:218-20, Negrier *et al., Blood* 1993*;* 81:690-5, Shetty *et al., Br J Haematol* 2007*;* 138:541-4

RNA interference



RNAi technology for gene silencing



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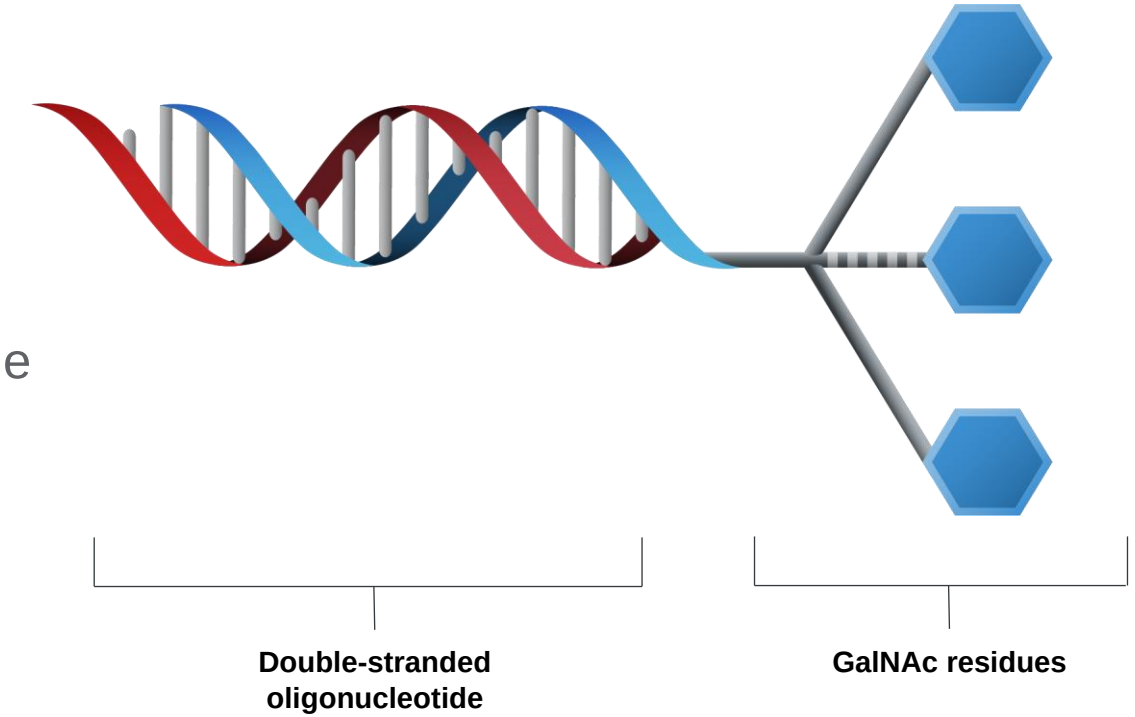
Fitusiran

- Small RNA molecule
  - 23 nucleotide
  - targeted to SERPINC1 transcript
- Attached to *N*-acetylgalactosamine (GalNAc) moiety
  - binding to the hepatocyte through the asialoglycoprotein receptor
- Administered SC monthly
- Fixed dosing



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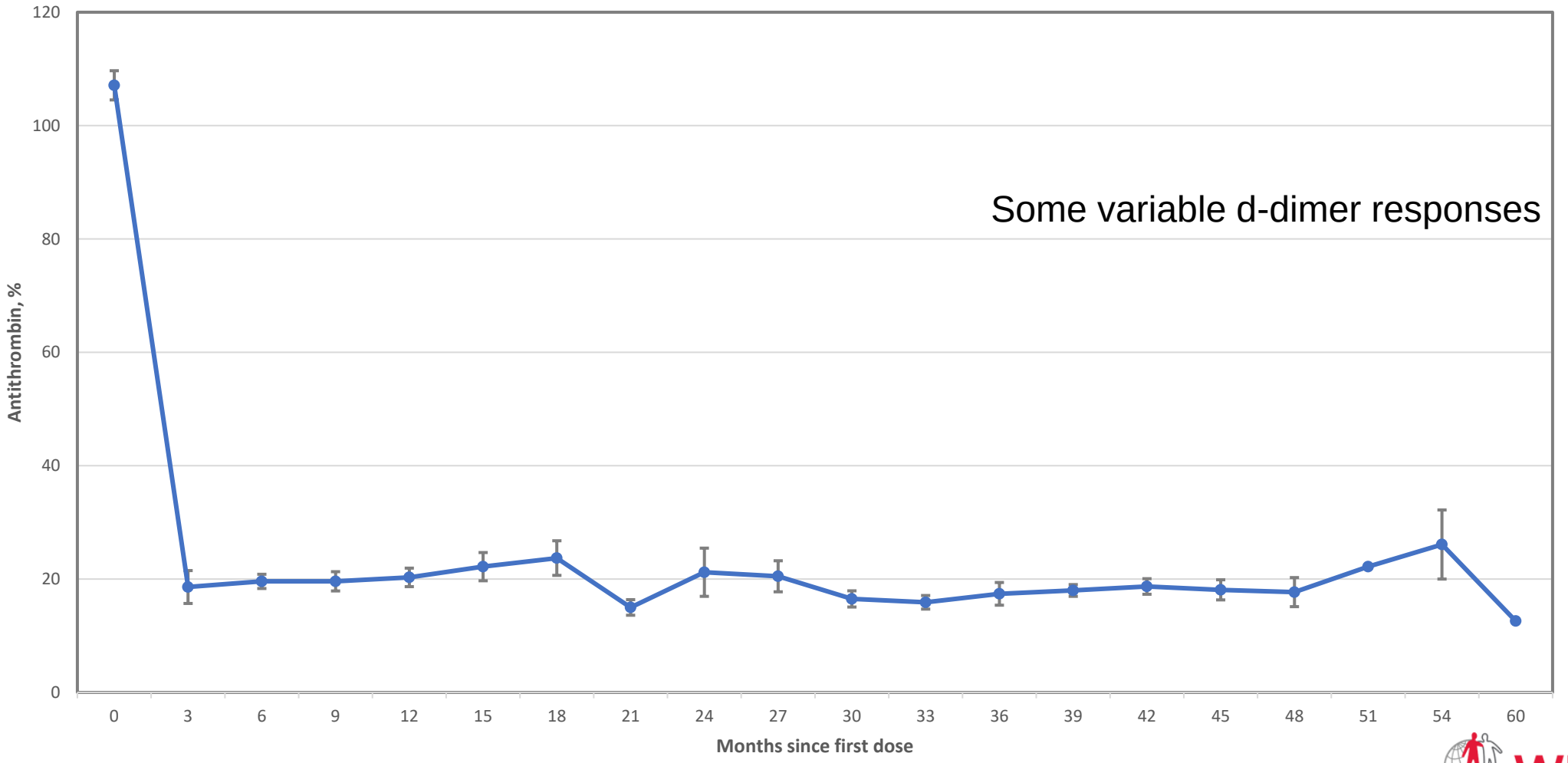
AT levels with Fitusiran - Phase 2 OLE



Pipe et al. ASH 2020

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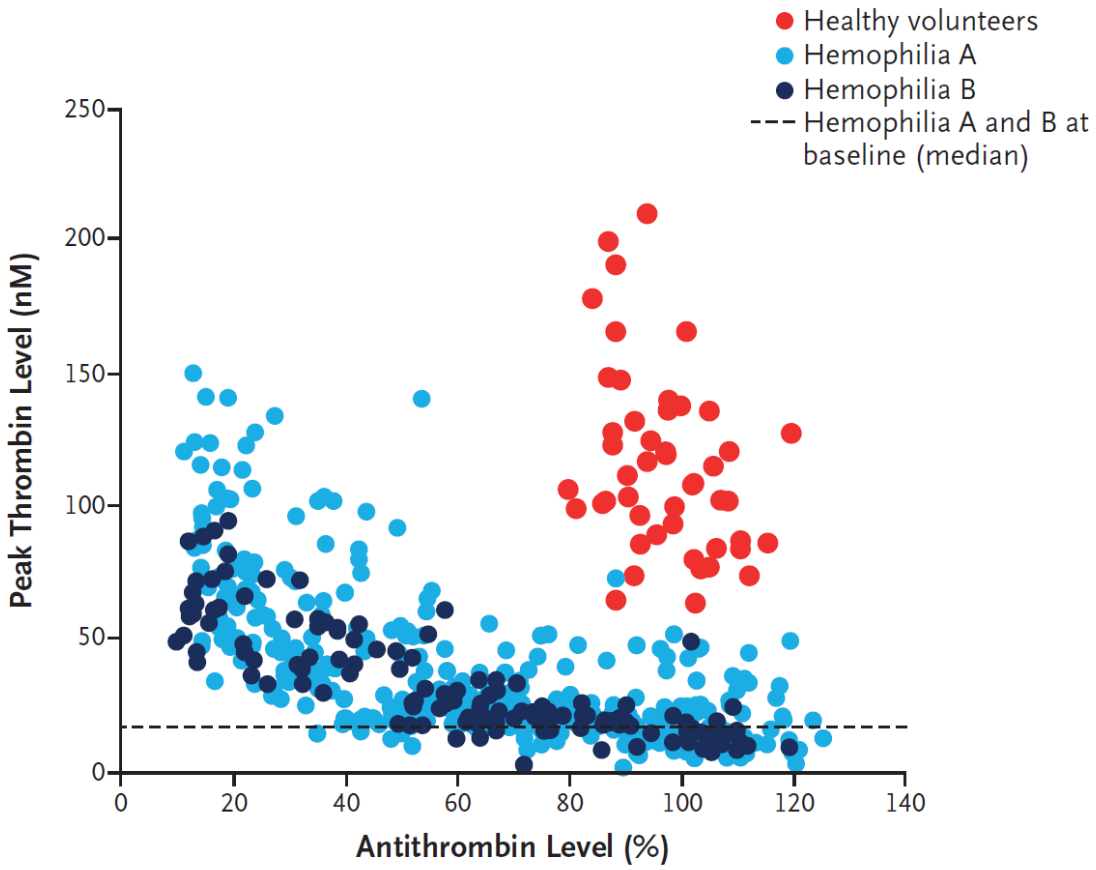
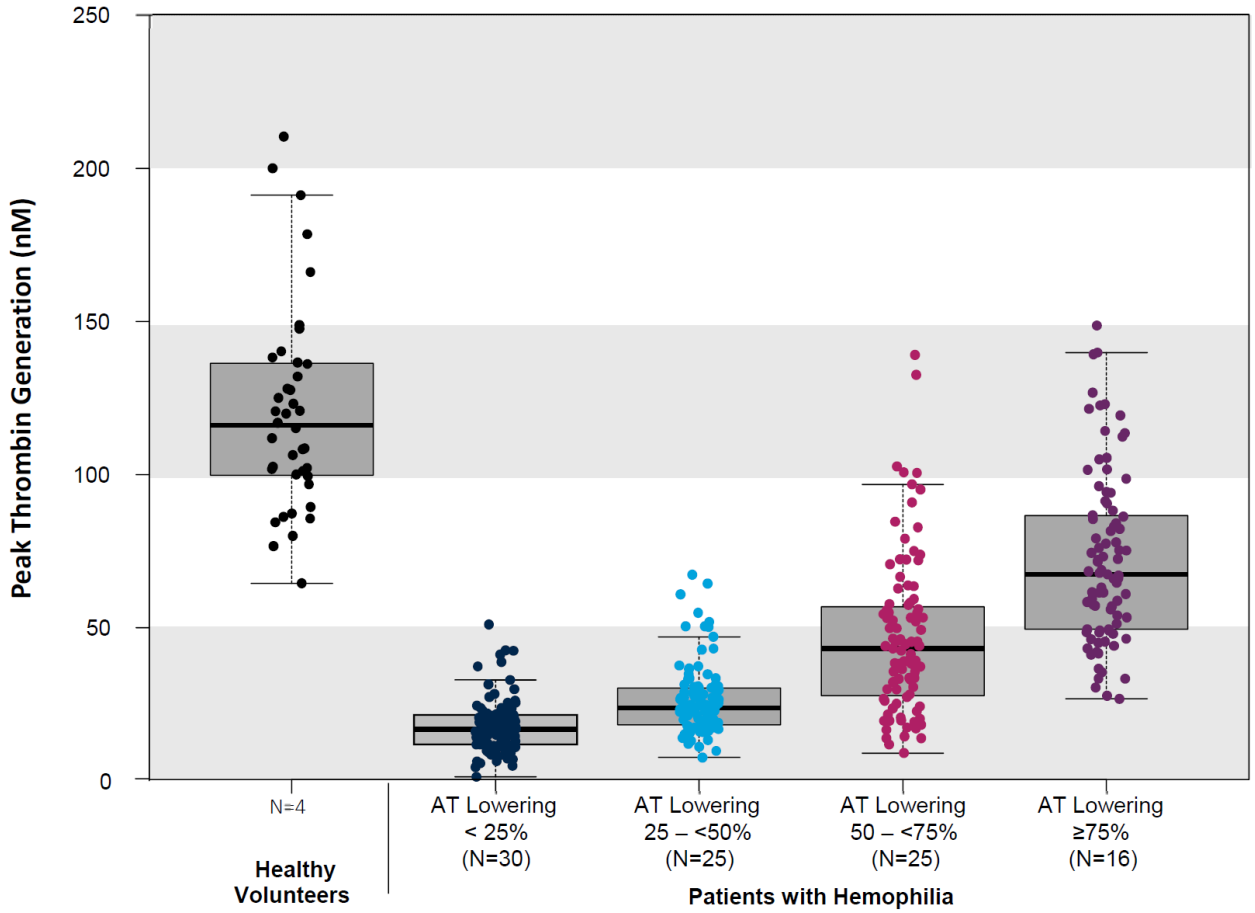
Thrombin generation





Pasi et al, *NEJM* 2017, 377:819-28

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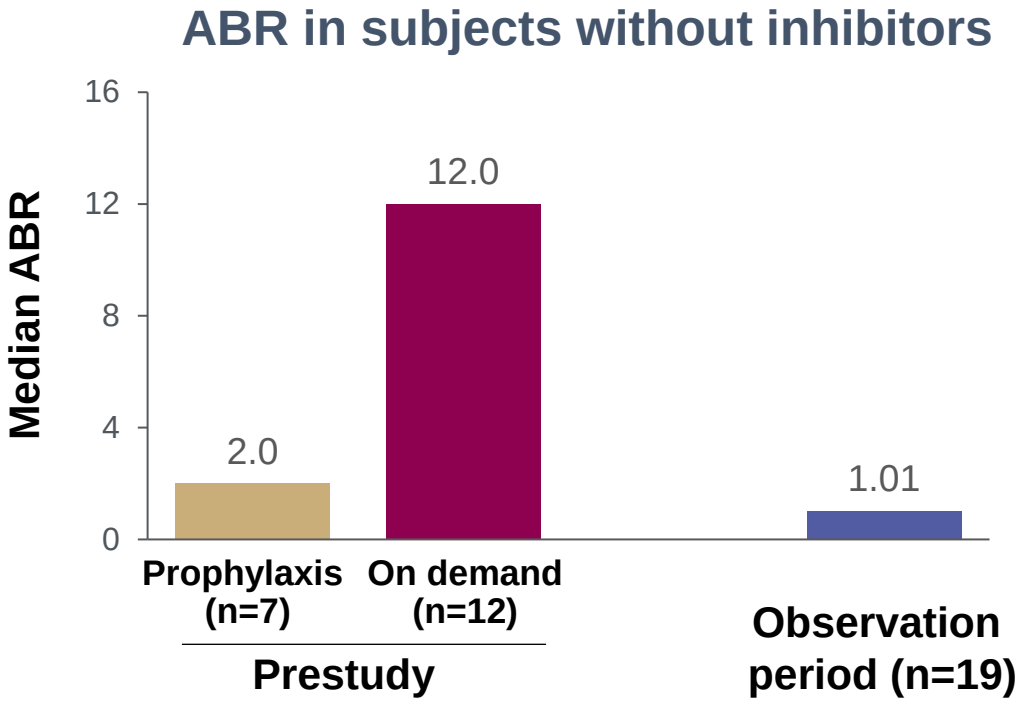
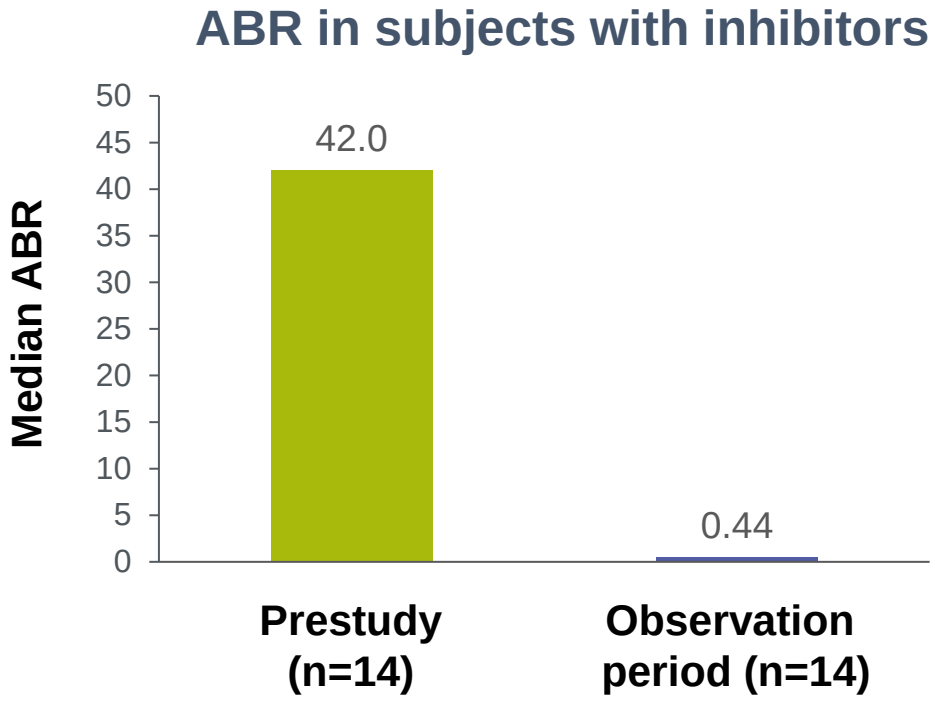
Fitusiran Phase 2 OLE : Bleeding Events

Overall median ABR of 0.84 during the observation period



Median duration in observation period:
36 months (range: 5-45 months)



Median duration in observation period:
28 months (range: 7-36 months)

Pasi et al, WFH Virtual Summit 2020

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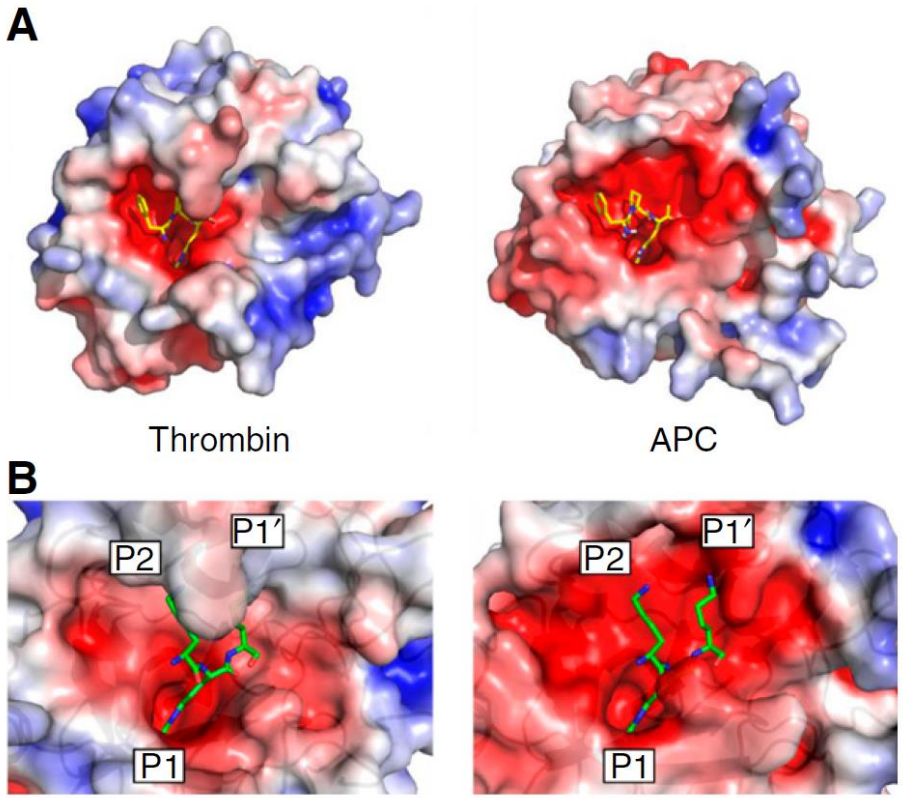
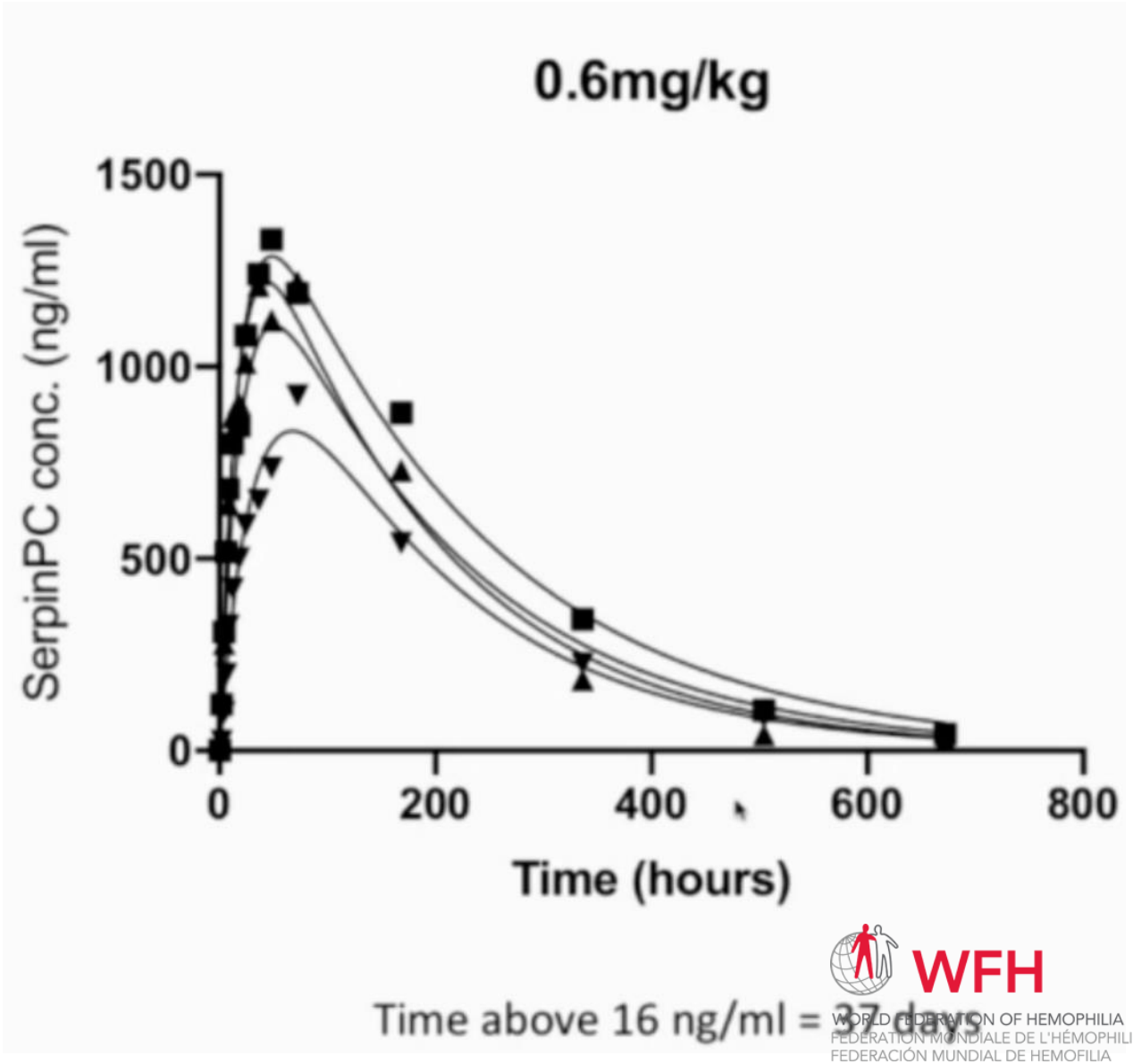
SerpinPC

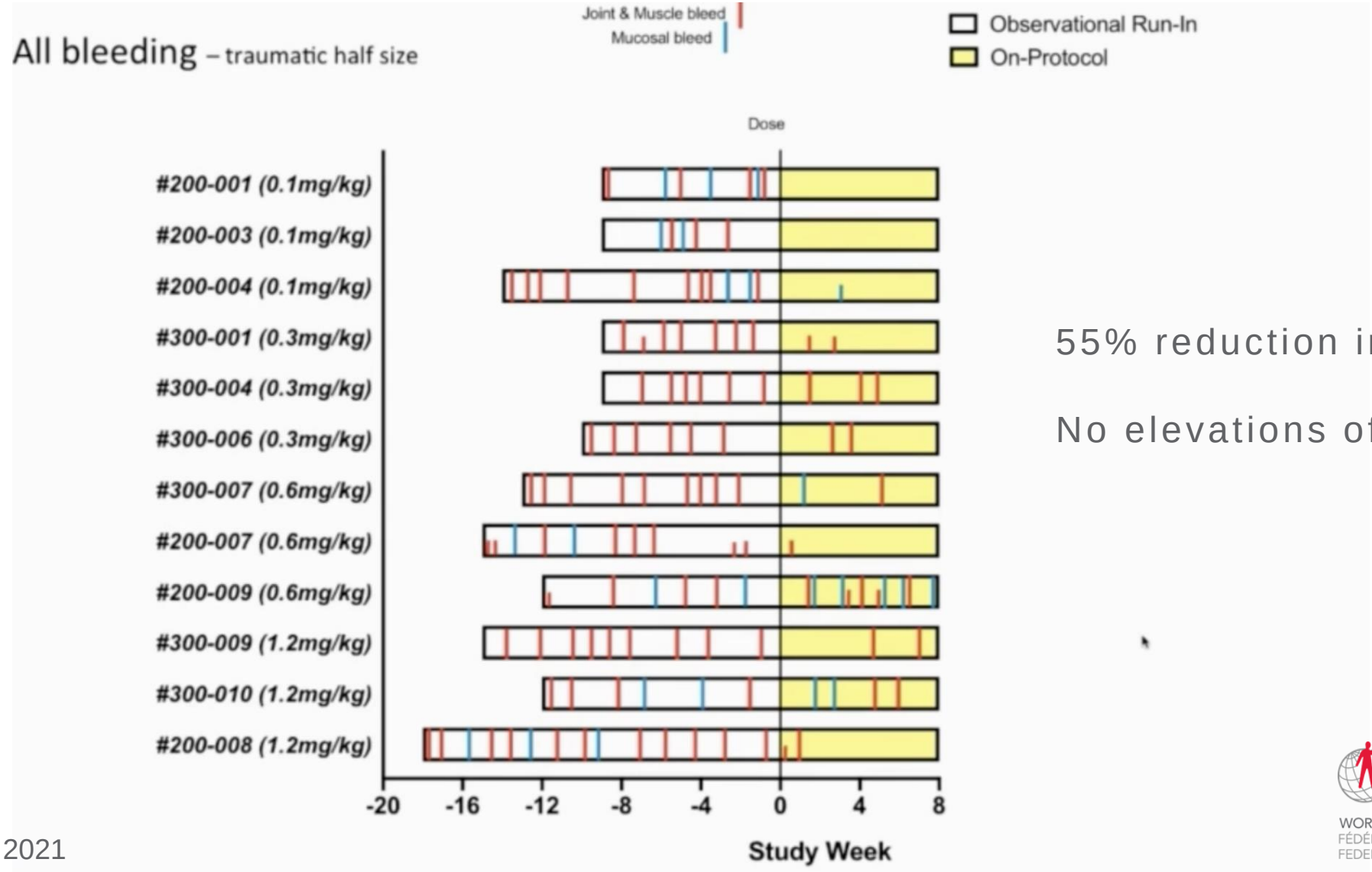


Specific design mutant alpha1-antitrypsin
KRK-alpha-AT
Covalent inhibition of APC (not PC)



Polderdijk et al, *Blood* 2017;129:105, Baglin et al EAHAD 2021

SerpinPC – bleed pattern after a single dose



55% reduction in bleeds

No elevations of d-dimer

Baglin et al, EAHAD 2021

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Safety and thrombosis risk

Risk mitigation strategies

BAY1093884 – thrombotic events

- 3 events
  - CVST (mild haem A with inhibitor)
  - Ischaemic stroke (severe haem A)
  - Retinal artery thrombosis (severe haem A)
- No event occurred in association with concomitant replacement therapy
- No association with level BAY1093884 exposure
- Events occurred at lower doses than PKPD predictions would suggest is required for efficacy
- Programme terminated

Ferrante et al, ISTH 2020

Concizumab – thrombotic AEs

- 3 thrombotic events
  - acute MI, renal infarct and VTE / PE
  - all recently treated with replacement therapy (FVIII, rVIIa)

- Issues
  - all had thromboembolic risk factors
  - all had concomitant treatment for breakthough bleeds
  - all had high(est) levels concizumab exposure

- Risk mitigation
  - lowest dosing for breakthrough bleeds
  - Concizumab exposure level to be used for dose adjustment

Seremetis et al, ASH 2020 poster 1796

Vascular Thrombotic Events

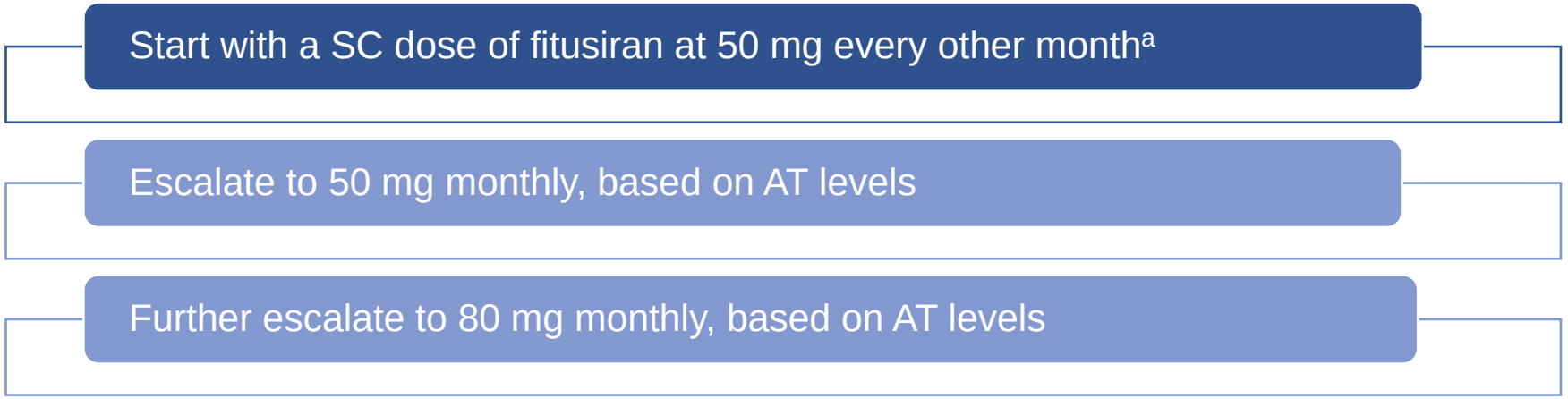
Vascular thrombotic event[c]	Patient characteristics		Medical history/comments	AT category
	Age range (years)	Haemophilia subtype and inhibitor status		
Cerebral infarct	>60	Haemophilia A patient without inhibitor	Well-controlled HIV, HCV, and prostate cancer status-post radical prostatectomy with recent prostate-specific antigen within normal limits	**<10%**
Cerebrovascular accident	30–40	Haemophilia A patient without inhibitor	Deep vein thrombosis (not identified at enrollment; a study exclusion criterion), diabetes, obesity, HCV and tobacco use	**<10%**
Spinal vascular disorder	20–30	Haemophilia A patient with inhibitor	Suspected thrombosis involving a spinal injury	**<10%**
Cerebral venous sinus thrombosis	20–30	Haemophilia A patient without inhibitor	Concomitant factor use in excess of the current bleed management guidelines in fitusiran clinical studies. Event initially diagnosed and treated as a subarachnoid haemorrhage and resulted in a fatal outcome	**10–20%**
Atrial thrombosis	20–30	Haemophilia B patient with inhibitor	Concomitant use of BPA in excess of the current bleed management guidelines in fitusiran clinical studies	**10–20%**

- ~293 patient-years of exposure
- Incident rate of vascular thrombotic events per 100 patient years vs AT level
  - <10%, 5.91
  - 10–20%, 1.40
  - >20% 0

Negrier et al, EAHAD 2021

Dose revisions

Based on PK/PD modelling and clinical data



Negrier et al, EAHAD 2021

Conclusions

- Significant potential for new therapeutic approach in haemophilia and rare bleeding disorders
  - differentiated approach with infrequent, subcutaneous dosing that could change disease management by restoring haemostasis
  - potential to address haemophilia A and B, all patient groups, including inhibitors
  - global public health issues and opportunities
  - potential simplicity

- Problems
  - risk of thrombosis
  - risk mitigation possible but loss of simplicity of dosing and monitoring

- Efficacy data needed from new dosing protocols

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THANK YOU

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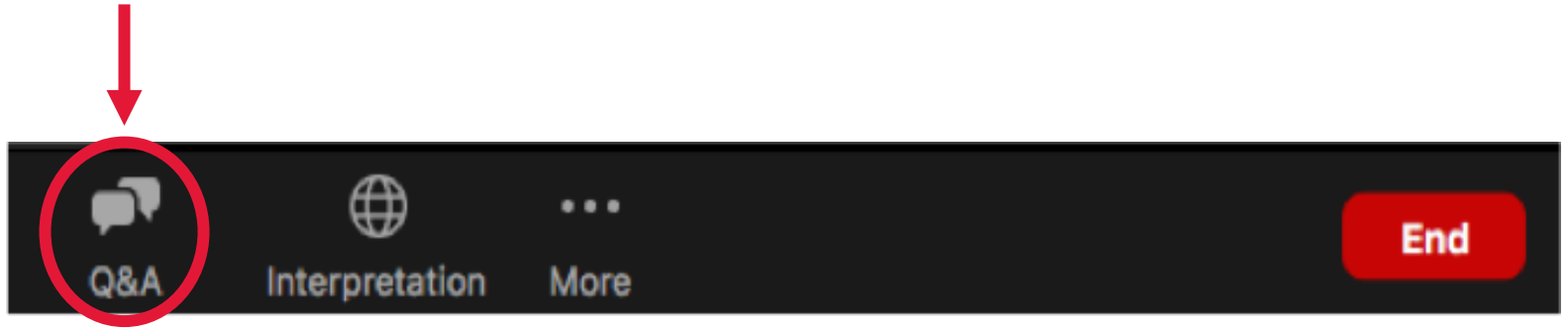
QUESTION & ANSWER

Please submit your questions in the Q&A box



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This webinar was part of a series. The next sessions will be taking place on the following dates:

- **Tuesday, July 27, 2021 from 8AM to 10AM ET**
- **Tuesday, October 12, 2021 from 8AM to 10AM ET**
- **Thursday, December 16, 2021 from 8AM to 10AM ET**

Registration will be open soon.

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COMPASSION to ACTION
Together we can

THANK YOU!

¡GRACIAS!

MERCI!

شكرا

СПАСИБО

STAY SAFE!

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