Hypo-coagulation disorders

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Etiology of coagulation disorders

According to the heredity:

- Herediraty – defect of synthesis
  - dysproteinemias
- acquired - defect of synthesis
  - increase turnover
    - consumption
    - antibodies
    - loss

According to the phenotype:

- bleeding disorders
- thrombotic states

Etiology of hereditary bleeding disorders

- Vessel wall (Ehler-Danlos, Rendu-Osler)
- trombocyto-
  - penia (TAR, Wiskot-Aldrich, Grey platelet sy)
  - patia (Glanzmann, Bernard- Soulier)
- **plasma coagulation (focus on today):**
  - hemophilia
  - von Willebrand disease
  - defects of the others plasma coagulation factors
  - hyperfibrinolysis: defect of alpha2-antiplazmin, PAI-1 ?

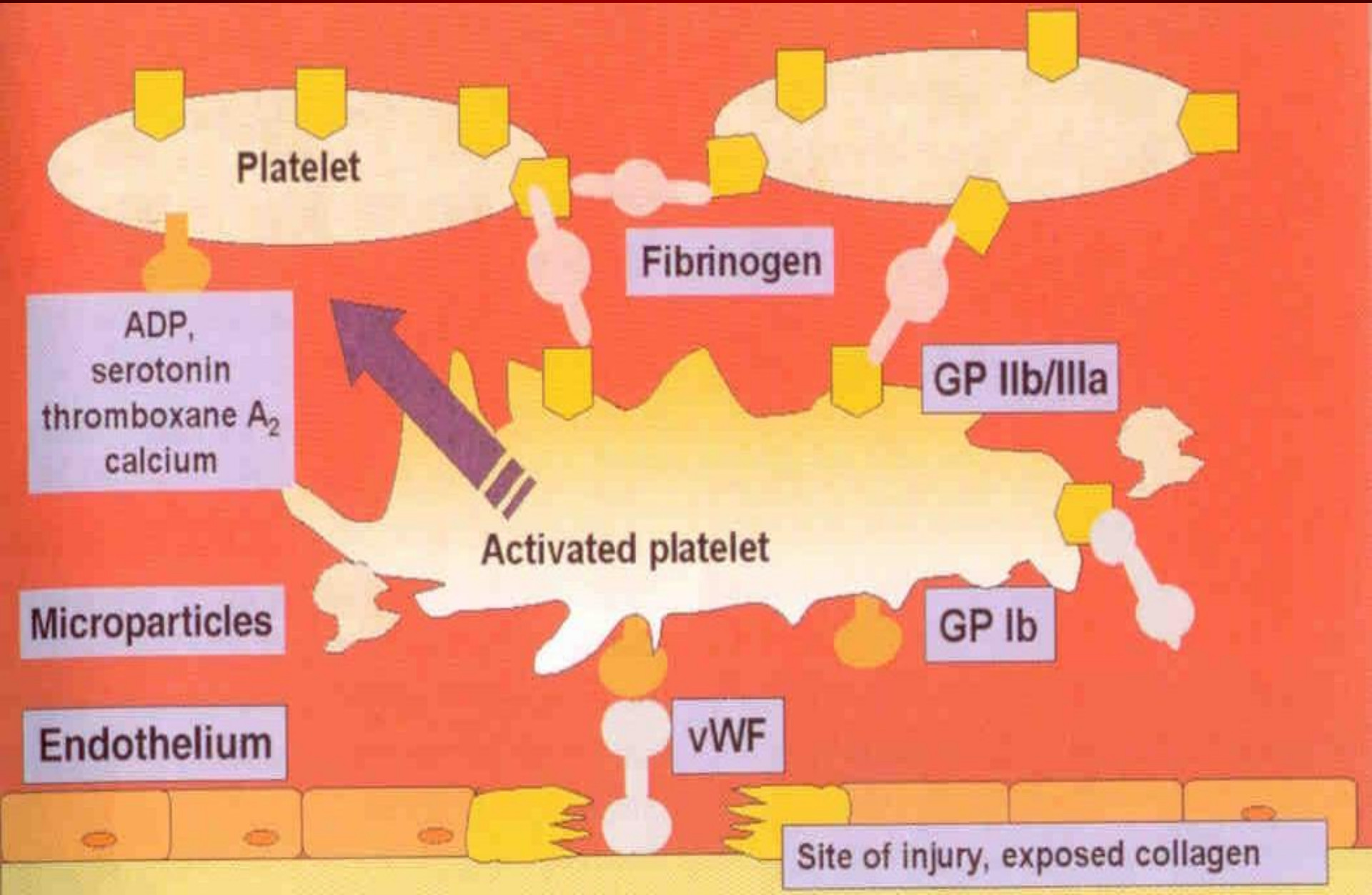
Hemostasis

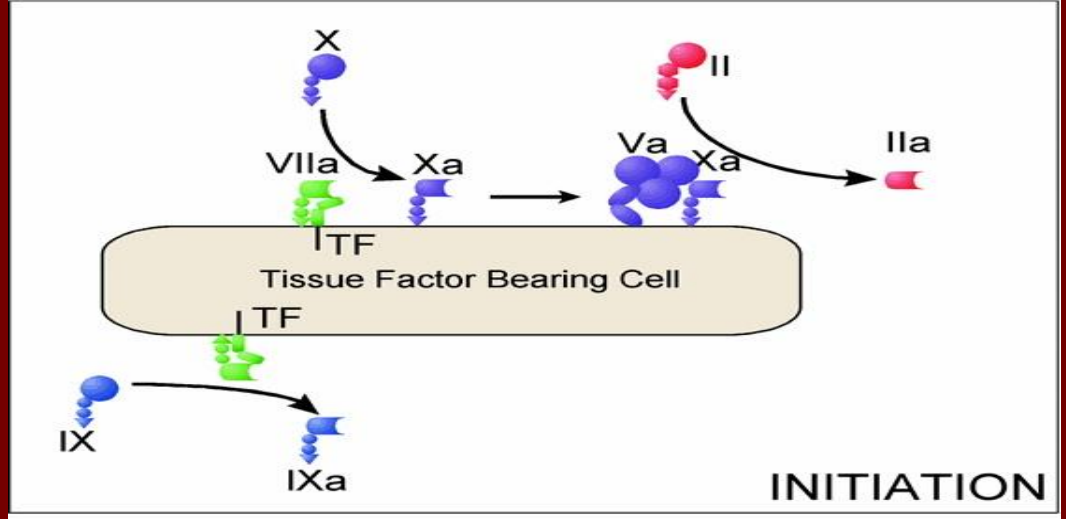
- **primary**
  - vasoconstriction — immediately
  - platelets adhesion — seconds
  - platelets aggregation — seconds to minutes
    minuty
- **secondary (coagulation)**
  - coagulation ff. Activation — seconds to minutes
  - Fibrin formation — minutes
- **fibrinolysis**
  - activation — minutes
  - blood clot lysis — hours

Bleeding manifestation

	prim. hemostasis	coagulopathy
petechias	typical	seldom
deep hematomas	seldom	typical
joints	seldom	typical
mucosal	spontaneous	after trauma
delayed	seldom	typical
from wounds	typical	minimal
bleed start	promptly	delayed

Primary hemostasis

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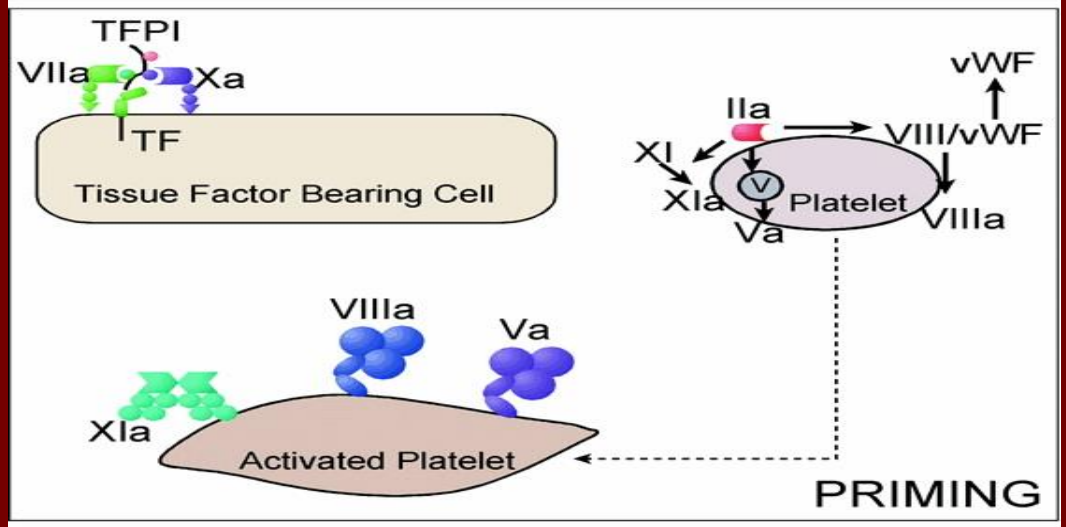
VIIa
Xa
TF
Tissue Factor Bearing Cell
TF
IX
IXa
II
Va
Xa
IIa
INITIATION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Tissue Factor Bearing Cell
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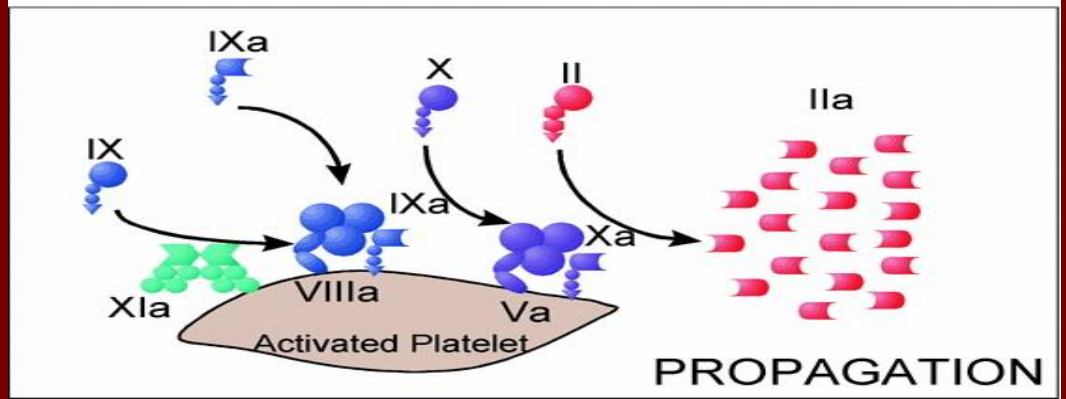
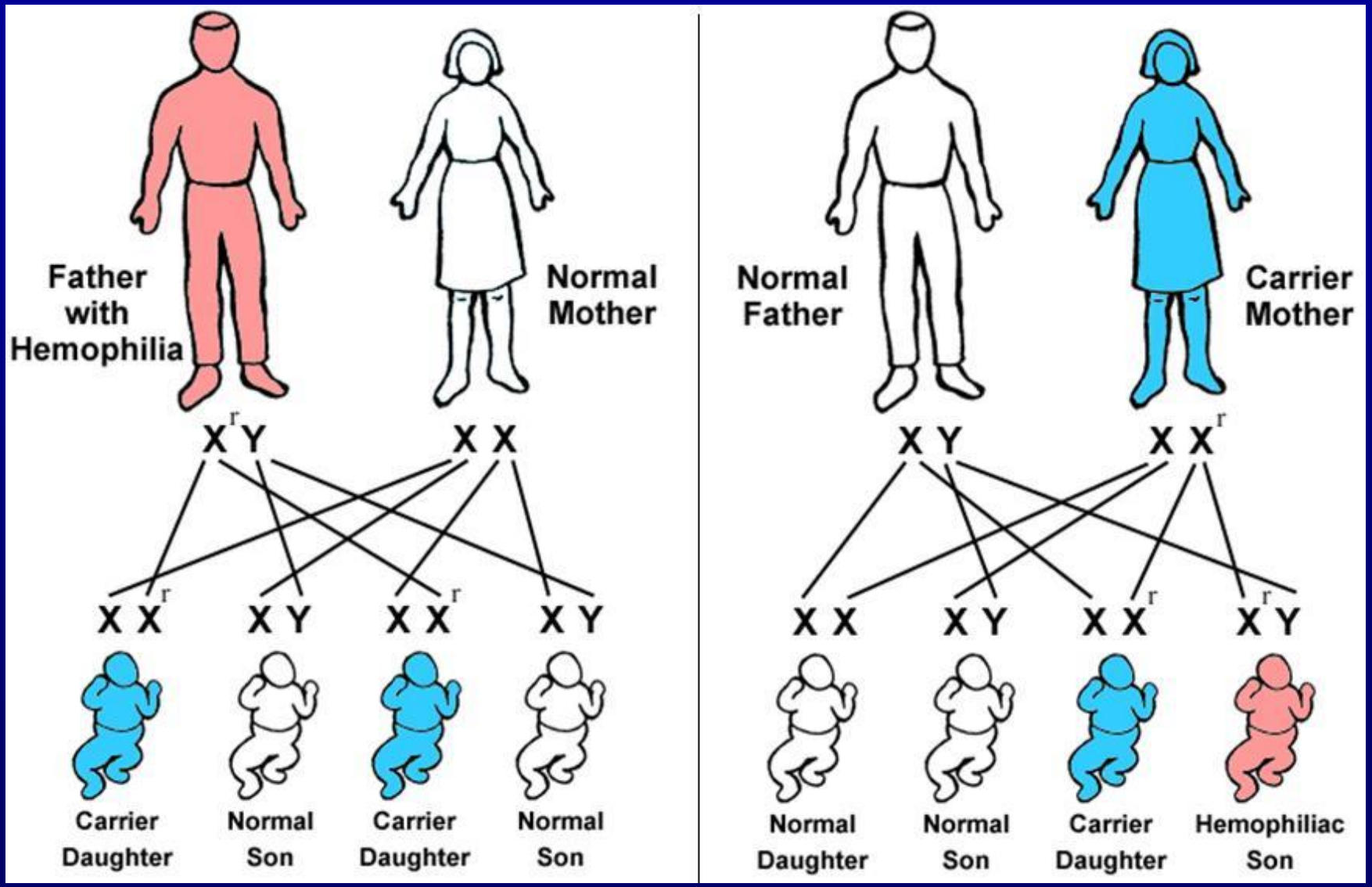
XIa
V
Platelet
Va
VIIIa
VIIIa
Va
XIa
Activated Platelet
PRIMING


IXa
X
II
IIa
IX
IXa
Xa
VIIIa
Va
XIa
Activated Platelet
PROPAGATION

Laboratoty tests of hemostasis

- **Coagulation: aPTT, PT, fibrinogen**, (TT)
  - Specific factors assays
- **Primary hemostasis:**
  - CBC - thrombocytopenia
  - Bleeding time, PFA-100, VWF:RCo
  - Assay of platelet function:
    - Aggregation
    - Retraction
    - Flowcytometry
    - Electron microscope
- Thrombelastometry
- Thrombin generation assay

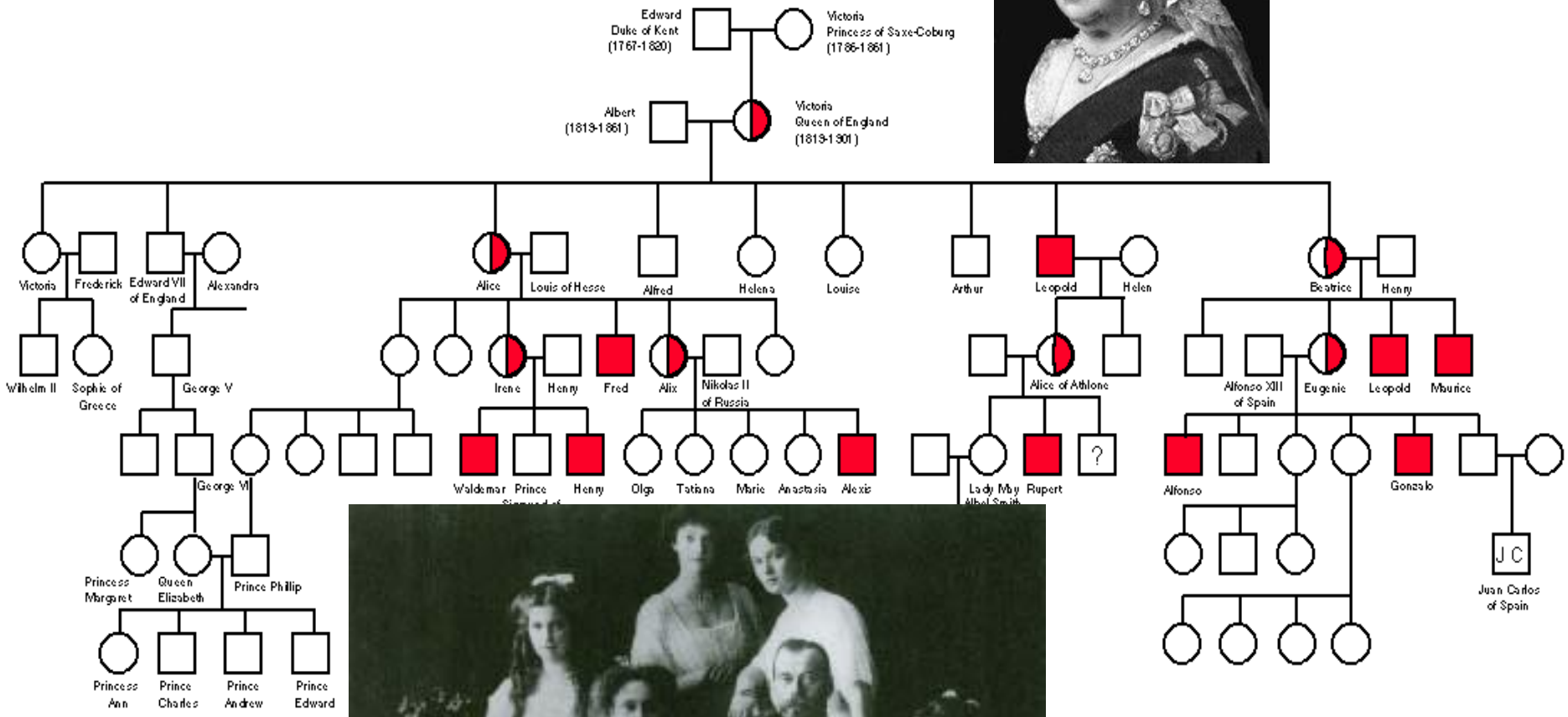
Hemophilia – X-recessive heridity



Hemophilia - prevalence

- Sporadic phenotype (25 - 30%)
  - Women carrier only:
    - Mostly without bleeding symptoms
  - New mutation
- Hemophila A (FVIII) 1/5000 – 10000 boys
- Hemophila B (FIX) 1/30000-50000 boys

Hemophilia – the royal disease



Hemophila A, B - diagnosis

- **bleeding: - joints, muscles, deep bruises**
- **severe < 1% FVIII / FIX**
  - spontaneous bleedeng frequent (1x monthly)
- **moderate 1 - 5% FVIII / FIX**
  - spont. bleeding seldom, after injury, surgery
- **mild 5 - 40% FVIII / FIX**
  - bleeding after injury, surgery

Hemophila - diagnosis

	hemophila	von Willebrand disease
• aPTT	↑	↑ - N
• PT (Quick)	N	N
• Bleeding time	N	N ↑↑
• PFA-100	N	↑↑
• assay	FVIII/FIX	VWF, FVIII

Hemophila – prenatal diagnosis

- Chorion villus sampling:
  - at 11–13 gestation week
- Amniocentesis:
  - at 15–18 gestation week
- 40% of severe hemophilia A:
  - **Inversion of intron 22**
- Foetal cell-free DNA in maternal blood:
  - from gestation week 7 – 9
  - 4% inconclusive results
- Preimplantation genetic diagnosis
  - a single cell is removed from a two-day zygote (8 cells)

Hemofilie A – léčba: stimulace uvolnění endogenního FVIII

- **DDAVP** - 0,3 μg / kg i.v. á 12 - 24 hod., max. 5 dnů
  (150 - 300 μg i. n.)

  - **elevace: - VWF 2- 4x** $t_{1/2}$ 5 - 10 hod.
  - FVIII 2- 4x $t_{1/2}$ 8 - 12 hod.

- Výchozí FVIII ≥ 15 %, aby byla šance dosáhnout hemostatické hladiny FVIII 40-50 %

DDAVP 1-desamino-8-D-arginin vasopressin

- Increase of VWF 2-3x:
  - releasing from endothelial cells
  - monocytes » secretion of PAF » VWF from endothelial cells
- Increase of FVIII 2-3x:
  - direct releasing from the site of synthesis
- Dosage:
  - 0,3 μg/kg i.v., s.c.,
  - 300 μg (<50 kg 150 μg) i. nasálně:
  - á 12 - 24 h.
  - max. 5 days
- For haemophilia A with FVIII:C ≥ 15 %
  - to have chance to achieve haemostatic level about 40-50 %

Hemophila treatment – substitution of FVIII/IX (plasma derived, recombinant)

- **FVIII:C 1 IU / kg increase 2% t2 = 12 h**
- **FIX:C 1 IU / kg increase 1% t2 = 18 h**

Prophylaxis:

- Primary: after 1st joint bleed and till age 2 years
- Secondary: started later
- Temporary: after surgery, severe bleeding

On demand:

- When bleeding has occured
- Perioperative substitution

Hemophilia - prophylaxis

Goal: to keep factor VIII/IX plasma level > 1 - 2%

Dosage:

- HA: FVIII 25 – 40 IU/kg 3 x weekly
- HB: FIX 25 – 40 IU/kg 2 x weekly
- Tailored:
  - 50 IU/kg 1 x weekly
  - 30 IU/kg 2 x weekly
- **Low: 25-35 IU/kg 1x weekly – lower risk of inhibitor**
  - Start before the first joint bleeding

Effect - lower risk:

- Life-threatening bleeding
- Joint bleeding – joint demage

Hemophilia – treatment of bleeding

(WFH – Guidelines for the Management)

no resource constraint	**FVIII (FIX)**	**resource constraint**
joints:		
40- 60%		10 – 20%
1 – 2 days, sometimes longer		
muscles:		
40 – 60%		10 – 20%
2 – 3 days, sometimes longer		
Musculus iliopsoas:		
80 – 100% (60-80%)	1 – 2 days	20 – 40% (15 – 30%)
30 – 60%	3 – 5 days	10 – 20%
prophylaxis		
hematuria:		
50% (40%)	3 – 5 days	20 – 40% (15 – 30%)
Deep laceration		
50% (40%)	5 – 7 days	20 – 40% (15 – 30%)

Hemophilia – treatment of bleeding

(WFH – Guidelines for the Management)

no resource constraint **FVIII (FIX)** **resource constraint**

- **CNS:**
  - 80 – 100% (60 – 80%) day 1 – 7
  - 50% (30%) day 8 – 21
  - 50 – 80% day 1 – 3
  - 30 – 50% day 4 – 7
  - 20 – 40% day 8 –14 till day 21
- **GIT:**
  - 80 – 100% (60 – 80%) day1 – 6
  - 50% (30%) day 7 – 14
  - 30 – 50% day 1 – 3
  - 10 – 20% day 4 – 7
- **Surgery**
  - 80 – 100% (60 – 80%) during surgery
  - 60 – 80% (40 – 60%) day 1 – 3
  - 40 – 60% (30 – 50%) day 4 – 6
  - 30 – 50% (20 – 40%) day 7 - 14
  - 60 – 80% (50 – 70%)
  - 30 – 40% day 1 – 3
  - 20 – 30% day 4 – 6
  - 10 – 20% day 7 - 14

Hemophilia – dental surgery - extraction

During extraction and next 6-12 h:

- **Desired plasma level:**
  - **FVIII 50-100%: FVIII 50 IU / kg**
  - **FIX 50-80%: FIX 80 IU / kg**

- **+ antifibrinolytic drug p.o. 8 - 10 days**
  - **Tranexamic acid (Exacyl)**
    - **3 x 25 mg / kg or 4 x 15-20 mg / kg /day**

Ageing hemophilic population – co-morbidities

	Dosage	Desired level FVIII/IX (IU)
ASA therapy	FVIII 25-40 IU/kg alternate day FIX 25-50 IU/kg 2-3x weekly	> 5%
Dual antiplatelet therapy	FVIII 15 IU/kg á 12 h FIX 15 IU/kg á 12 h	> 25%
Thrombolysis Therapeutic dose of heparins	FVIII 40 IU/kg + 20 IU/kg á 12 h FIX 80 IU/kg + 30 IU/kg á 12 h	>80% >50%
Warfarin therapy	FVIII 15 IU/kg á 12 h FIX 15 IU/kg á 12 h	> 25%
Trombocytopenia < 30 000 / μl	FVIII 10 IU/kg daily FIX 20 IU/kg alternate day	> 5%
Liver biopsy	FVIII 50 IU/kg, FIX 70 IU/kg FVIII, FIX 25 IU/kg á 12 h	during procedure >70% next 2 (4) days > 50%

**Mannucci PM. Blood 2009:5256-63* **Schutgens REG. Haemophilia 2009:952-58*
**Mauser-Bunschoten EP. Aging with haemophilia 2007*

Hemophila – gene therapy

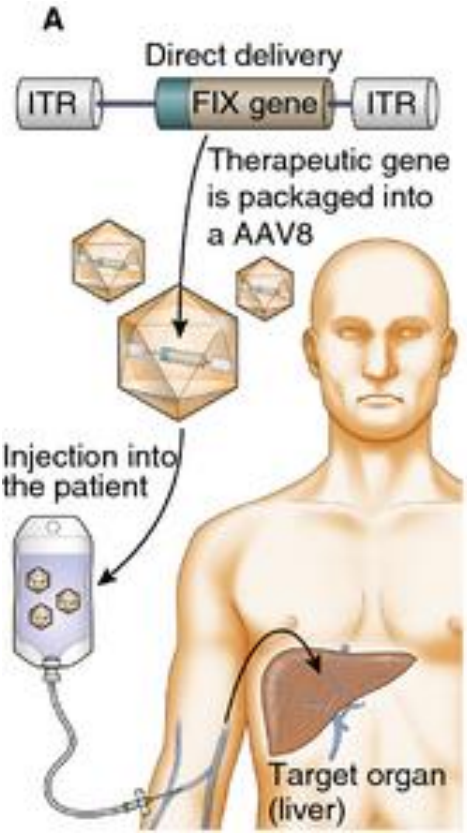
restoration of FVIII/FIX synthesis by gene transfer:

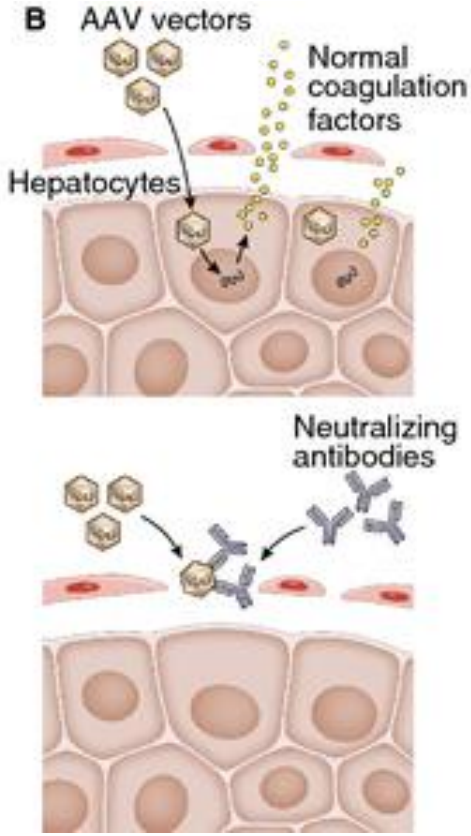
- **In vivo:**
  - i.v., i.m., s.c.
- **Ex vivo:**
  - to the cells ex vivo and their implantation

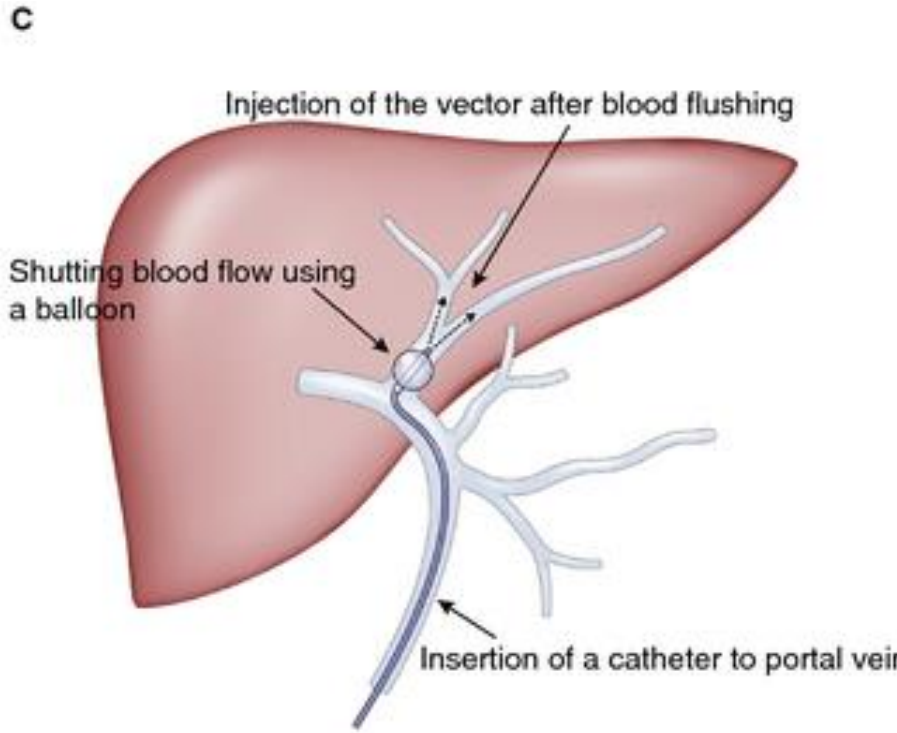
Vectors:

- **Viral:**
  - retrovirus - s.c.: tumor risk
  - adenovirus - i.m.: virus elimination by immune systeme
  - adeno-asociated virus – i.m.: only small gene – hemophilia B
- **Non-viral -** use naked plasmid DNA transferred by:
  - liposoms, nanoparticles
  - elektroporation
  - direct injection

Gene therapy approaches for hemophilia by direct administration of AAV8

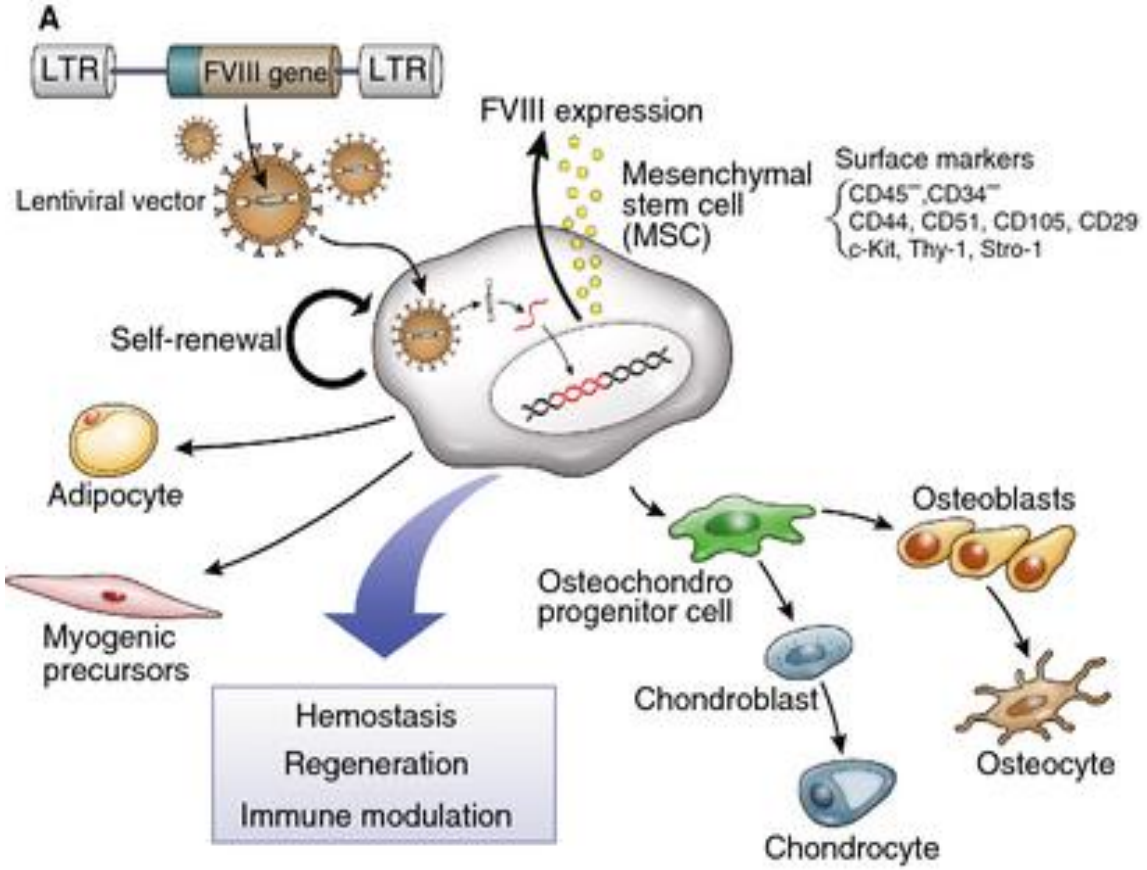






Journal of Thrombosis and Haemostasis
pages S133-S142, 19 JUN 2015 DOI: 10.1111/jth.12926
http://onlinelibrary.wiley.com/doi/10.1111/jth.12926/full#jth12926-fig-0001

Local cell-based therapy for hemophilic arthropathy by MSCs expressing coagulation factor



Journal of Thrombosis and Haemostasis
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http://onlinelibrary.wiley.com/doi/10.1111/jth.12926/full#jth12926-fig-0002

Gene therapy – haemophilia B

- for severe: FIX < 1 %
- AAV8
- 1/3 of patients has má neutralizing antibodies
- hore than 50 patients from 2011:
  - $2x10^{11}$ vg/kg - $2x10^{12}$ vg/kg
  - for 3-4 years FIX plasma level 1-6 %
  - ↑ LT week 6-12
    - destruction of hepatocytes by T-lymphocytes
  - Padua mutant FIX (p.R338L):
    - 5-10x ↑ coagulation activity
    - FIX plasma level 18-80 %

*Dolan G.Eur J Haematol. 201799(Suppl.87): 3-9
*Nathwani AC. Human Gene Therapy 2017;28: 1004-12

Gene therapy – haemophilia A

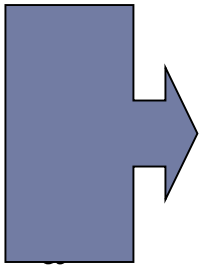
- larger gene: 7 kb
- from 2015
- AAV5, 15 patinets:
  - Deletion of B-domain (4,4 kb)
  - 7: $6x10^{13}$ vg/kg: after 1 year FVIII > 50 %
  - 6: $4x10^{13}$ vg/kg: after 1 year FVIII > 50 % 3x, 3x > 5 %
  - 3: $6x10^{12}$ vg/kg a $2x10^{13}$ vg/kg: without increase of plasma FVIII
- Lentivirus
  - Possibility to transfer of large gene

Nathwani AC. Human Gene Therapy 2017;28: 1004-12

Concentrate with modified FVIII / FIX molecule

Prolongation of plasma half-life:

- Pegylated
- Fusion with albumin
- Fc fragment fusion



Approved for treatment

EHL rFVIII:

- $T_{1/2}$ 1.5 x ↑ (18-19 h)

EHL rFIX:

- $T_{1/2}$ 3-5x ↑ (60-90 h)

Ortopaedic procedures in management of hemophilic arthropathy

- Synoviorthesis (synovectomy):
  - chemical (small joints)
  - radioactive (elbows, knees, ankles)
- Surgical synovectomy:
  - elbows, knees, ankles
- Total joint arthroplasty:
  - knees, hips
- Arthrodesis:
  - Joint is fused without further motion, without pain

Hemophila – FVIII/FIX inhibitor

- allo-antibodies
  - neutralized factor clotting activity
  - mostly class IgG

- 1 **Bethesda U:**
  - amount of inhibitor that destroys half the factor in the mixture of normal and patient´s plasma after 2 h incubation

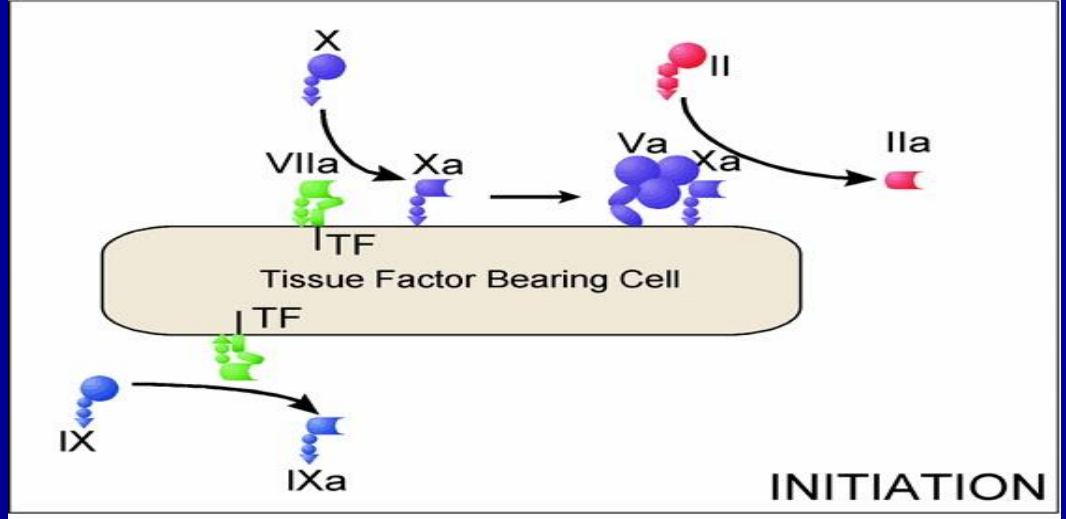
- 20-30% patients with severe HA, < 4% with HB
  - median detection is 10-15th ED (exposure day)

Inhibitor type

responder	"Low"	"High"
• Response to infused FVIII/IX	without rise of inhibitor titre	rise of inhibitor titre
• Inhibitor titre	< 5 BU/ml	> 5 BU/ml
• Incidence	cca 1/3	cca 2/3

Treatment of bleeding episodes with inhibitor

- High dose of FVIII/IX concentrates (< 5 BU/ml)
- aPCC - activated prothrombin complex concentrate (FVIIa, FII, FIX, FX) **FEIBA®**
  - 50-100 IU/kg á 6-12 h
  - Limitation: maximum 200 IU/kg per day
- rFVIIa **NovoSeven®**
  - 90 µg/kg á 2-3 h
  - **Single dose 270** µg/kg
- Plasmapheresis with immunoadsorption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VIIa
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Tissue Factor Bearing Cell
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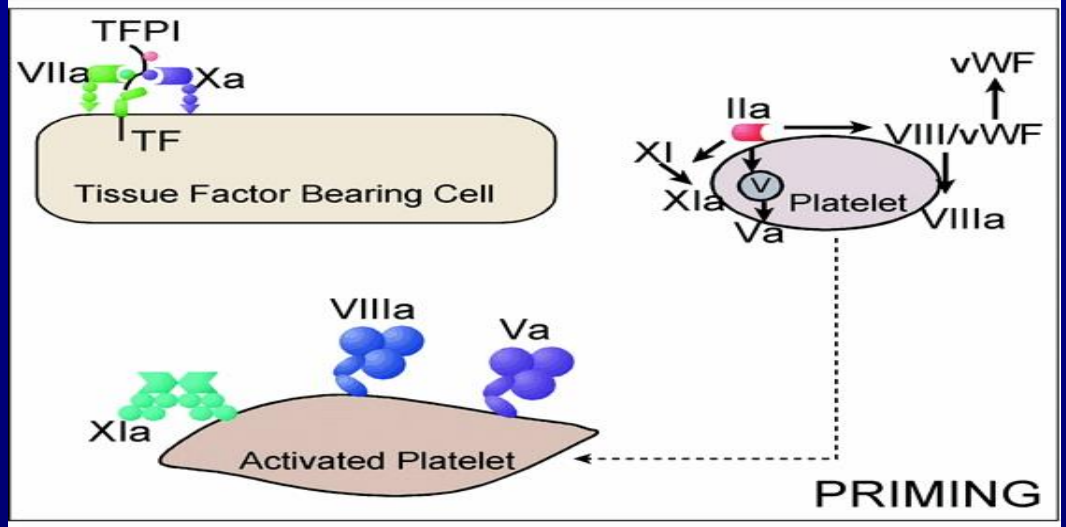
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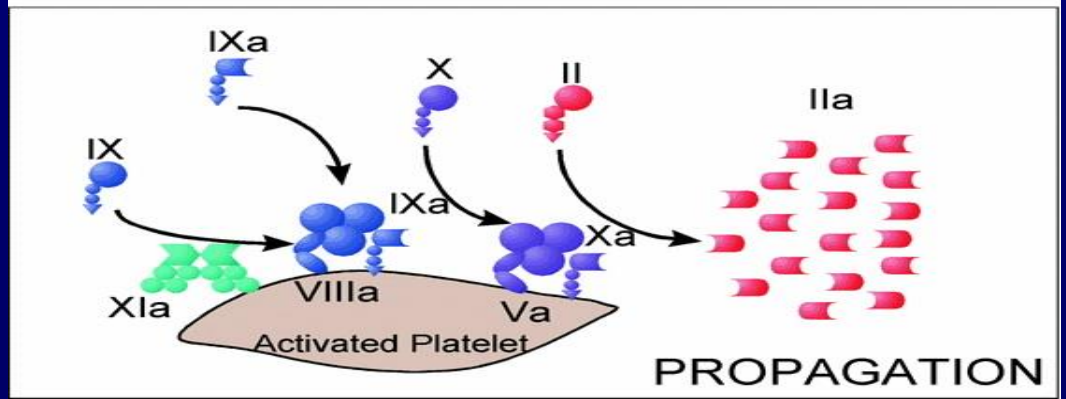
IXa
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PROPAGATION

Immune tolerance induction - ITI

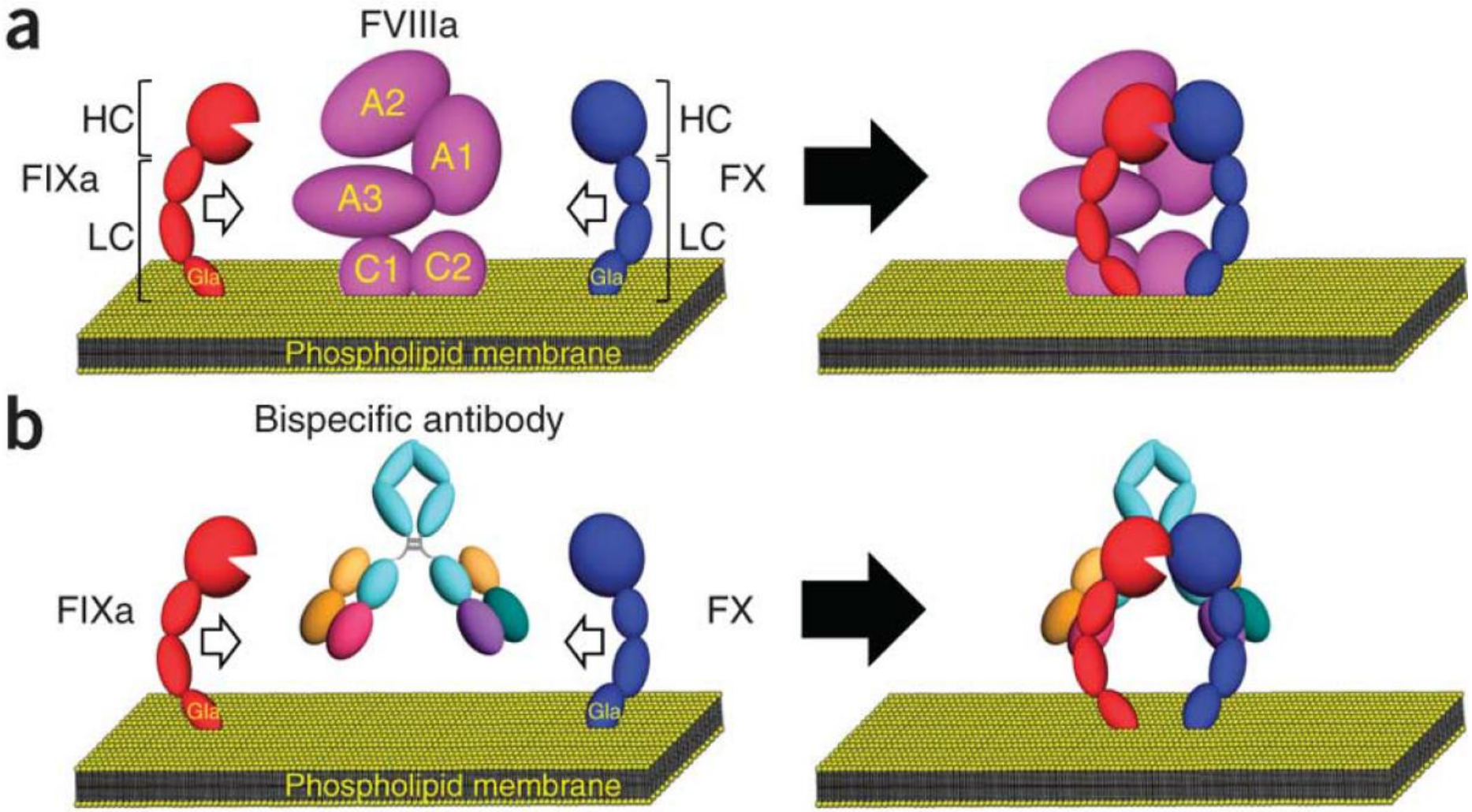
- **Bonn protocol:**
  - FVIII/IX 200-300 IU / kg / day
  - High responders
  - Inhibitor eradication: 85%

- **low dose protocol:**
  - FVIII/IX ≤ 50 IU / kg / day (3 x weekly)
  - Low responders
  - Inhibitor eradication: 67%

- **Malmö protocol:**
  - Plasmapheresis with immunoadsorption of IgG
  - Immunosupresive treatment – cyclophosphamide
  - FVIII/IX substitution – neutralize inhibitor and rise level > 30%
  - Inhibitor eradication: 50%
-

FVIII-mimetic function of bispecific antibody

as a cofactor promoting the interaction between FIXa and FX



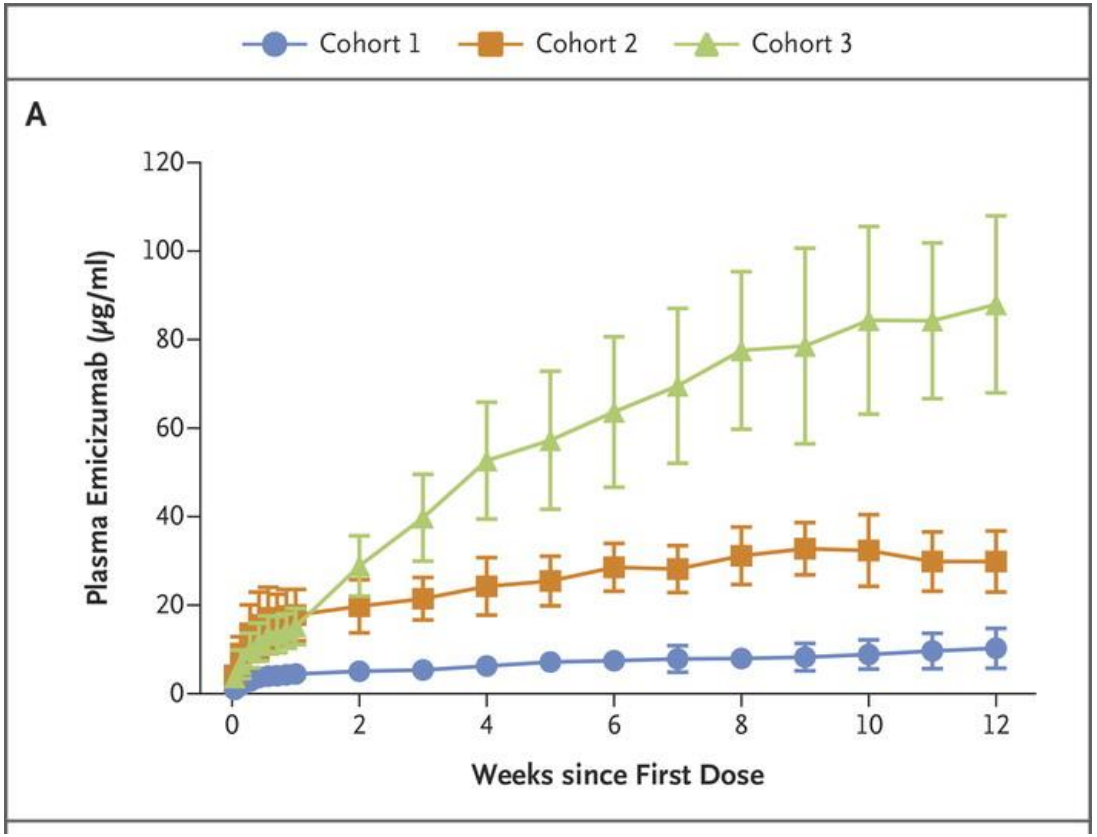
Kitazawa T et al, Nature Med 2012; 18: 1570–1574 (2012)

Emicizumab – dosage and plasma level

- Dosage 1x weakly **s.c.**:
  - group 1: 0,3 mg/kg
  - group 2: 1 mg/kg
  - group 3: 3 mg/kg

„Trough level“ week 12:

- 10 µg ~ 3 % FVIII
- 30 µg ~ 9 % FVIII
- 90 µg ~ 27 % FVIII



- 30-50 µg ~ 10-15% FVIII

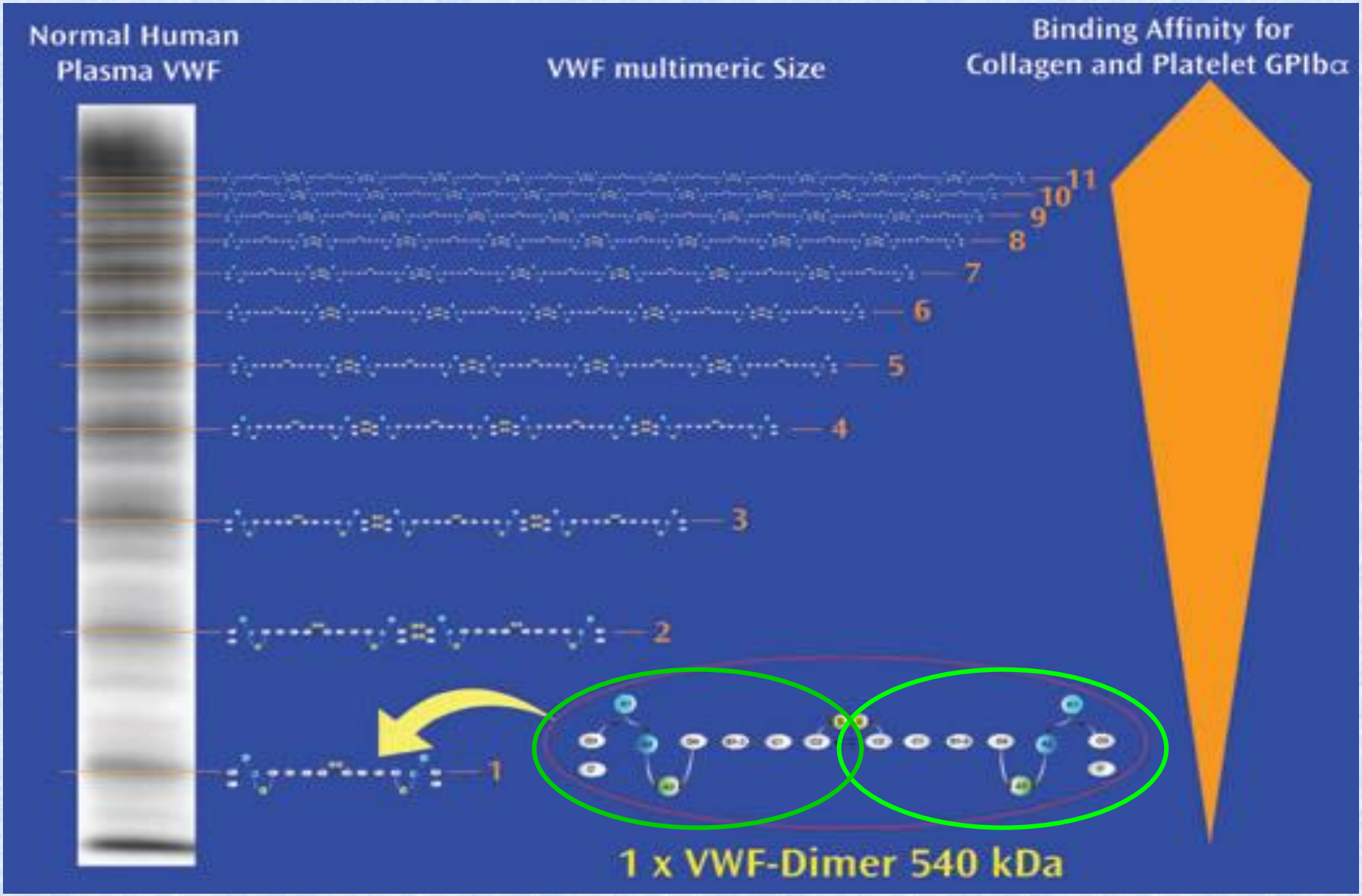
- Recommended dosage: 3 mg/kg s.c. 1x weakly for 4 weeks
- next 1,5 mg/kg 1x weakly or 3 mg/kg á 2 weeka or 6 mg/kg á 4 weeks

**Shima M. et al. N Engl J Med 2016;374:2044-53*

von Willebrand disease

- is the most common hereditary bleeding disorder
  - Low level of VWF: 1%
  - Disease 1.000-3.000 / 1.000.000
    - Severe type 3 / 1.000.000
- caused by quantitative or qualitative defect of VWF
  - multimeric structure glycoprotein
  - synthesized in endothelial cells and megakaryocytes
  - carrier of FVIII
  - promoting
    - platelet adhesion to subendothelium
    - platelet aggregation

Multimer structure + electrophoretically separated bands



*Reininger AJ. Haemophilia 2008;14 (Suppl.5):11-26

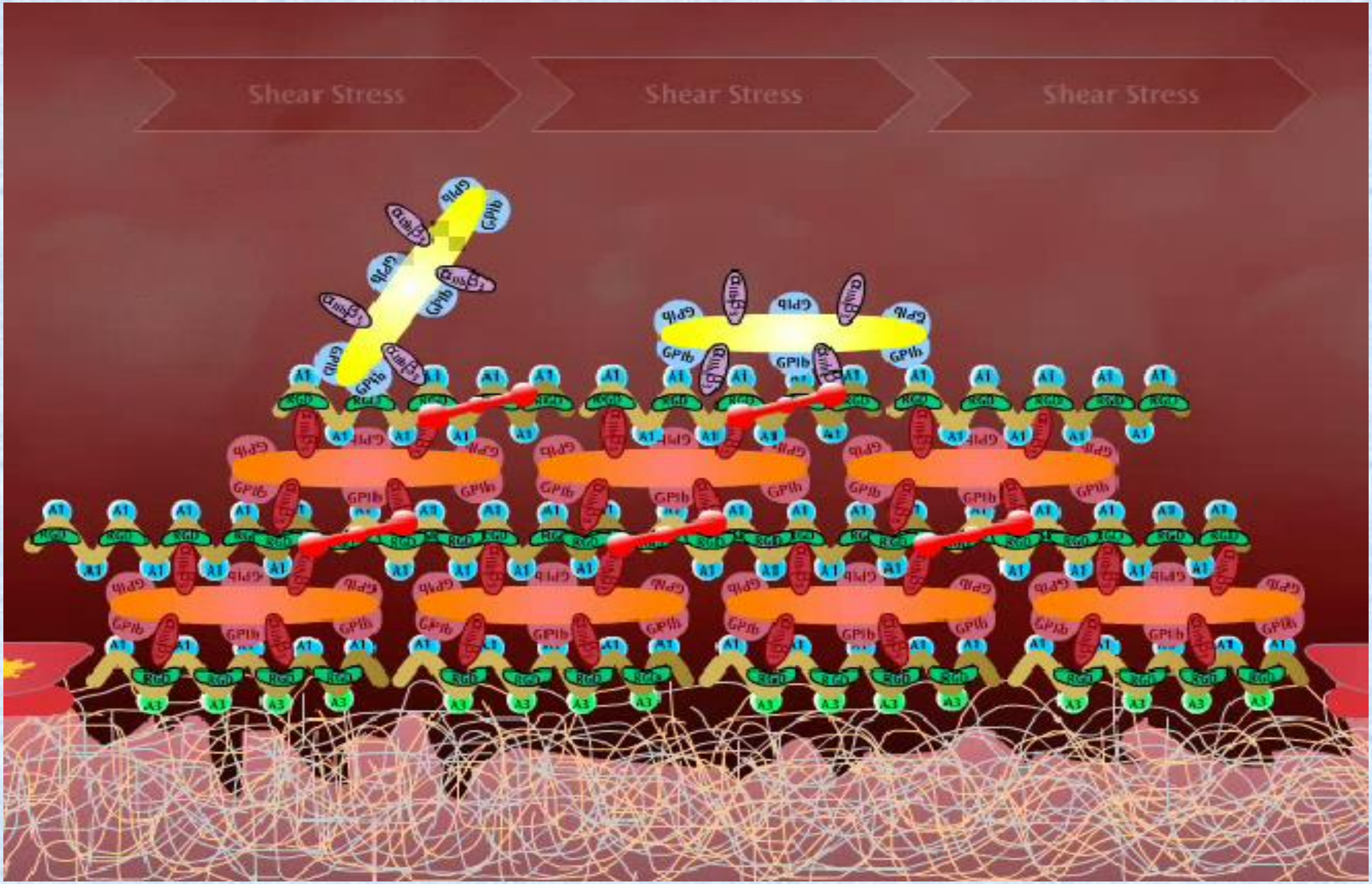
Shear induced VWF changes and imobilization

No shear 35 Dyn/cm^2

**Reininger AJ. Haemophilia 2008;14 (Suppl.5):11-26*

**Siedlecki ChA. Blood 1996;88:2239-50*

Primary platelet clot



Classification of von Willebrand disease

**Sadler JE, Thromb Haemost 1994; 71: 520-525*

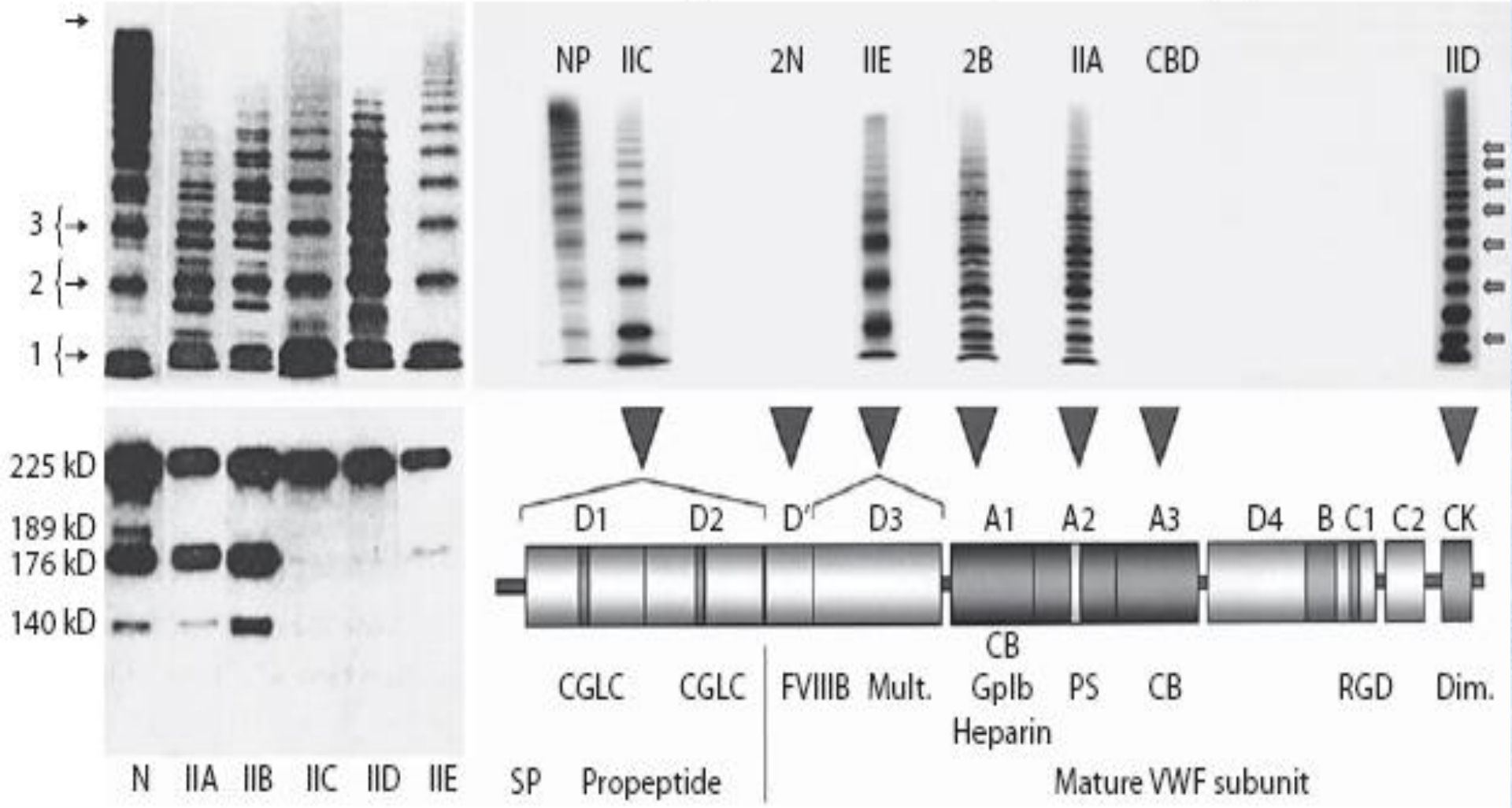
- type 1 – partial quantitative deficiency, AD
- type 2 – qualitative defects, AD, AR
  - 2A – decreased VWF-dependent platelet adhesion and deficiency of HMW multimers, AD
  - 2B – increased affinity for platelet GPIb, AD
  - 2M – decreased VWF-dependent platelet adhesion without selective deficiency of HMW multimers, AD
  - 2N – decreased binding affinity for FVIII, AR
- type 3 – virtually complete deficiency of VWF, AR

Changes in the classification of VWD

**Sadler JE,J Thromb Haemost 2006; 4: 2103-2114*

- **VWD is not restrict to VWF gene mutation**
- **VWD type 1 includes partial quantitative deficiency:**
  - HMWH multimers of VWF are decreased only relatively
  - normal ratio of functional activities compared with VWF:Ag

SSC-ISTH classification of VWD 2A subtypes



*Schneppenheim R.Semin Hematol 2004;42:15-28

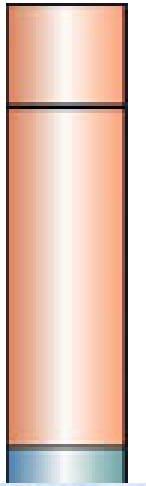
*Gadisseur A.Acta Haematol 2009;121:128-38

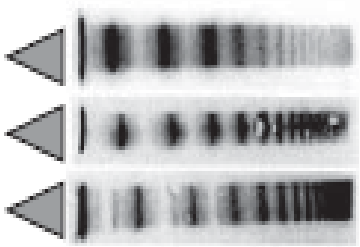
Laboratory diagnosis of VWD

- **Screening tests:** sensitivity
  - platelets (↓type 2B)
  - aPTT < 30%
  - bleeding time < 40%
  - PFA100 85 - 90%
- **Specific tests:**
  - **VWF:Ag, VWF:RCo + VWF:CBA, FVIII:C**
- **Discriminating tests:**
  - multimeric analysis of VWF, RIPA, VWF FVIII binding capacity, VWFpp
- **Molecular diagnosis**
- **Assay of platelet VWF**

Role of the VWFpp/Ag ratio

- VWFpp is released after VWF secretion
- half-life:
  - VWFpp: 2-3 h
  - VWF:Ag 8-12 h
- diagnosis of increased clearence:
  - in VWD
  - in acquired VWF defects



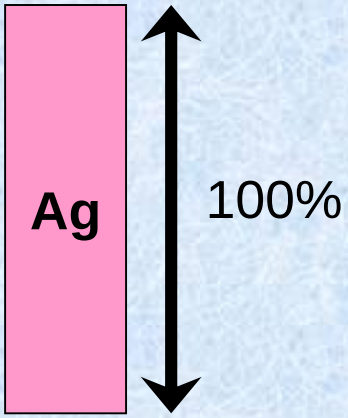


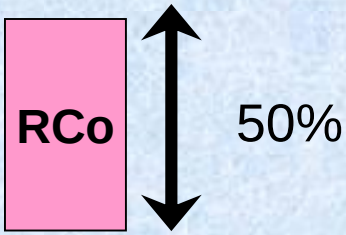
VWF domain	Multimers	Mutation	FVIII:C	VWF:Ag	VWF:RCo	VWF:RCo/ VWF:Ag	VWF:pp/ VWF:Ag	VWD type
		WT	78–135	71–119	62–113	0.93	1.0	–
		C1130R	13–15	12–22	7–13	N/↓	2–4	I/2E
		W114G	24	31	12	N/↓	2–4	I/2E
		R1205H	7–19	5–10	3–10	±1	>10	I/Vicenza

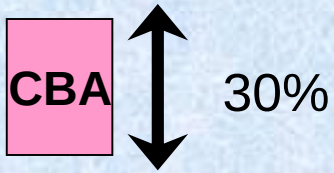
**Gadisseur A.Acta Haematol 2009;121:128-38*

Collagen binding assay - VWF:CBA

- more sensitive for HMW multimer deficiency:
  - type 2A ,2B: vWF:CBA / vWF:Ag < 0,5







**Favaloro EJ.Semin Thromb Hemost 2006;32:456-71*
**Favaloro EJ.Semin Thromb Hemost 2006;32:566-76*

A summary of the routine laboratory findings in the various types of VWD

type	RIPA	RCo	Ag	FVIII	RCo/Ag	CBA	CBA/Ag
1	N↓	↓	↓	N↓	N	↓	N
2A	↓↓	↓↓	N↓	N↓	↓< 0,7	↓↓	↓< 0,5
2B	N↑	↓	N↓	N↓	↓< 0,7	↓↓	↓ < 0,5
2M	N↓	↓	N↓	N↓	↓< 0,7	N ↓	N
2N	N	N ↓	N↓	↓	N	N	N
3	↓ ↓	↓ ↓	↓ ↓	↓ ↓	N↓	↓ ↓	N ↓

Diagnosis of von Willebrand disease

(SSC ISTH Subcommittee on vWF, 1996)

**Sadler JE, J Thromb Haemost 2005; 3: 775-777*

- confirm: a) mucocutaneous bleeding
  b) family history
  c) laboratory tests
  VWF: RCo, VWF:Ag < 2 SD (BG 0, non-0)

- possible:
  – without a) or b)

Grades of bleeding severity used in the IMS

**Tosetto A.Haemophilia 2008:14:415-22*

	Score			
Symptom	0	1	2	3
Epistaxis	No or trivial	Present	Packing, cauterization	Blood transfusion or replacement therapy
Cutaneous	No or trivial	Petechiae or bruises	Haematomas	Consultation
Bleeding from minor wounds	No or trivial	Present (1–5 episodes per year)	Consultation	Surgical haemostasis
Oral cavity	No or trivial	Present	Consultation only	Surgical haemostasis/blood transfusion
GI bleeding	No or trivial	Present	Consultation only	Surgery/blood transfusion
Tooth extraction	No or trivial	Present	Suturing or packing	Blood transfusion
Surgery	No or trivial	Present	Suturing or resurgery	Blood transfusion
Menorrhagia	No or trivial	Present	Consultation, pill use, iron therapy	Blood transfusion, hysterectomy, dilatation and curretage
Postpartum haemorrhage	No or trivial	Present, iron therapy	Blood transfusion, dilatation and curettage, suturing	Hysterectomy
Muscle haematomas	No or trivial	Present	Consultation only	Blood transfusion, surgery
Haemarthrosis	No or trivial	Present	Consultation only	Blood transfusion, surgery

Minimaly diagnostic criteria for clinicaly useful diagnosis of VWD - BS

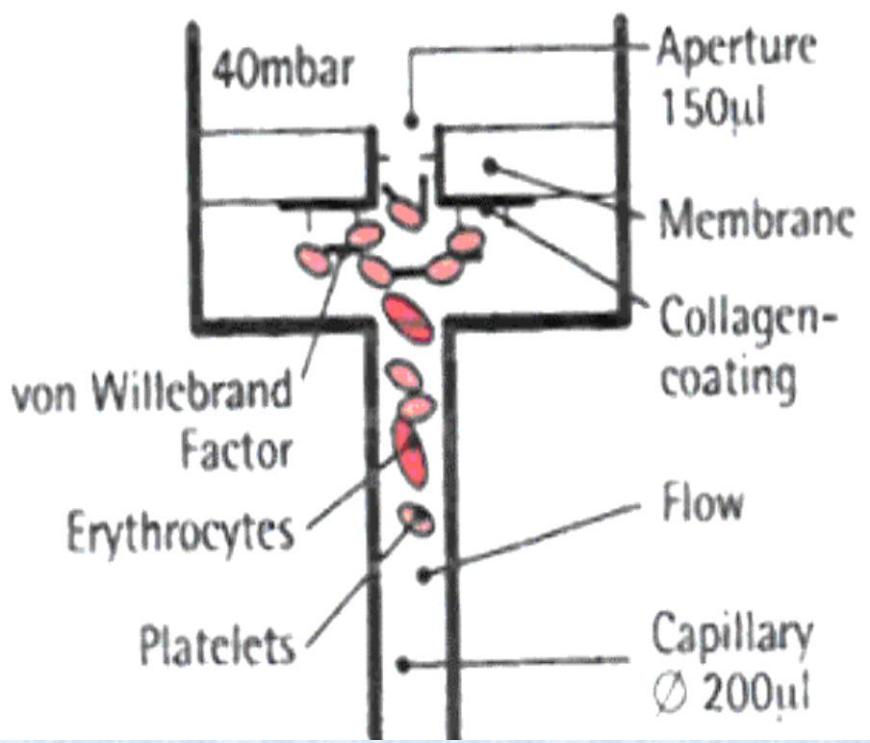
- **bleeding score:**
  - > 3 in men
  - > 5 in women
  - requirement for high BS is less stringent in children

**Rodeghiero F.2009;51st Congress of ASH,New Orleans*

The utility of the PFA-100 in the identification of VWD

Sensitivity:

- > 98% to VWD
  - types 2A, 2B, 2M, 3
- 50 – 100% to VWD type 1
  - 50% - not specified cut-off for vWF:Ag, RCo
- 85 – 90% overall



**Favaloro EJ. Semin Thromb Hemost 2006;32:537-45*

Minimaly diagnostic criteria for clinicaly useful diagnosis of VWD – tests of VWF

- MCMDM-1vWD cut off for percentil 2,5 (n=1166):
  - BG 0:
    - VWF:RCo 43%
    - VWF:Ag 44,4%
  - BG non-0:
    - VWF:RCo 54%
    - VWF:Ag 54%

- VWF:Ag or VWF:RCo:
  - < **40%**
    - **30 – 40%** only if BS is > 3 / 5 in men / females

**Rodeghiero F.2009;51st ASH,New Orleans*

- < **30%**

**Nichols WL. Haemophilia 2008;14:171-232*

VWD – therapeutic weapons

- **release of endogenous VWF:**
  - DDAVP
- **VWF substitution:**
  - pd concentrates containing WF/FVIII
  - platelet concentrates
- **other forms:**
  - antifibrinolytics
  - estrogens

DDAVP 1-desamino-8-D-arginin vasopressin

- Increase of VWF 2-3x:
  - releasing from endothelial cells
  - monocytes » secretion of PAF » VWF from endothelial cells
- Increase of FVIII 2-3x:
  - direct releasing from the site of synthesis
- Dosage:
  - 0,3 μg/kg i.v., s.c.,
  - 300 μg (<50 kg 150 μg) i. nasálně:
  - á 12 - 24 h.
  - max. 5 days

Efficacy of DDAVP in therapy of VWD

- type 1: v 90%
- typ 2: max. about 50%:
  - 2A (increase of proteolysis, t2: 2- 4 h.)
  - 2M (variable)
  - 2N (t2: 2- 4 hod.)
  - 2B (contraindication - progress of thrombocytopenia)
- typ 3: VWF/FVIII level < 5-10%
  - ineffective

Substitution of VWF/FVIII

- cryoprecipitate (FVIII/VWF 80 – 100 IU/1 TU)
- pd-concentrates of FVIII containing VWF:
  - variable content of HMW multimers of VWF
  - the highest ratio VWF:RCo/FVIII:
    - Haemate P®

– labeled:
  - VWF:RCo
  - FVIII:C

Comparison various pd FVIII/VWF concentrates

concentrate	VWF:RCo / FVIII	Recovery / IU / kg	t1/2 (h.)	FVIII IU / 1 mg
Haemate P®	2,4	2%	7	2 - 6
Fanhdi®	1,2	2%	14	2,5 - 10
Wilate®	0,9	1,5 - 2%	18 - 34	≥ 60
Willfact®	≥ 10	1,5 - 2%	8 - 14	≥ 50 VWF:RCo

High purity VWF concentrate (Wilfactin®, Willfact®)

- Increase of FVIII:C - rate 6% per 1 h
  - In acute bleed combination woth FVIII concentrate
  - In case of surgery 1st dose 12 h before surgery

- t2 VWF:RCo = 12 h.
- t2 FVIII:C = 17 h.

Recommended level of VWF:RCo and FVIII:C

bleeding type	desired level		duration of substitution
	VWF:RCo	FVIII:C	
major surgery	> 50%	> 50%	until healing (7 - 10 days)
minor surgery	> 30-50%	> 30-50%	until healing (1 - 5 days)
dental extraction	> 50%	> 50%	for 12 h
	+ antifibrinolytics		5 -10 days
bleeding episodes	> 30-50%	> 30-50%	until bleeding stops (2 - 4 days)
vaginal delivery	> 40-50%	> 40-50%	3 - 4 days

**Mannucci PM.Blood Transfus 2009;7:117-26*
**Nichols WL. Haemophilia 2008;14:171-232*
**Nordic Guidelines on VWD 2008*

Rare inherited coagulation bleeding disorders

- Heredity autosomal recessive
- Severe defects 1 / 1 000 000
  (homozygot, double heterozygot)
- dysfibrinogenemia (AD, bleeds or TEN or without)
- Factors deficiency:
  - fibrinogen, F II, V, VII, X, XI, XIII
- Fibrinolysis inhibitors deficiency:
  - α2AP, PAI,
-

Dysfibrinogenemia

- **Diagnosis:**
  - **↑ thrombin time and reptilase time**
  - **Antigen > functional activity (Clauss)**
- Fenotyp (mostly AD):
  - **Asymptomatic** 55% (Aα Arg 16 His)
  - **Bleeding** 25% (Aα Gly 17 Val)
  - **Thrombosis** 20% (Arg 554 cys)
    - To be include in screening of thrombophilia
- More than 300 mutations
- **Therapy:**
  - Bleeding: substitution of fibrinogen
  - Thrombosis: LMWH, kumarins
  - Abortions:
    - LMWH
    - substitution

Coagulation tests and BT in factor deficiency

Factor	PT	aPTT	TT	BT	remarks
A-fbg	↑	↑	↑	↑	fbg 0
Dys - fbg	↑	↑	↑	N	Fbg activity ↓, Fbg:Ag N, ↑ rept time
II	↑	↑	N	N	< 30 %
V	↑	↑	N	N - ↑	< 30 %
VII	↑	N	N	N	< 30 %
VIII	N	↑	N	N	< 30 %
VWD	N	↑ - N	N	↑ - N	FVIII: N - ↓, VWF:RCo < 40-50 %
IX	N	↑	N	N	< 30 %
X	↑	↑	N	N	< 30 %
XI	N	↑	N	N	< 40 %
XII	N	↑	N	N	< 40 %
XIII	N	N	N	N	↓ coagulum lysis
$_{\alpha 2}$AP	N	N	N	N	↓ coagulum lysis
PAI-1	N	N	N	N	↓ coagulum lysis

Prevalence of severe hereditary coagulation disorders

- Fibrinogen 1 : 1 000 000
- FII 1 : 2 000 000
- FV 1 : 1 000 000
- FVII 1 : 300 000 – 500 000
- FV+VIII 1 : 2 000 000
- FVIII (XR) 50 – 80 : 1 000 000 (not only severe)
- FIX (XR) 10 – 15 : 1 000 000 (not only severe)
- FX 1 : 1 000 000
- FXI 1 : 1 000 000 (Ashkenazi 8% heterozygotes)
- FXIII 1 : 1 000 000
- MvW (AD) 100 – 1 000 : 1 000 000 (not only severe)

Factor	Type of bleeding in severe defect	Abnormal assay	Desired level in bleeding / surgery	Treatment
fbg	umbilical, CNS, soft tissues	PT, aPTT, TT	0,5 - 1 g / l	Fibrinogen
II	Soft tissues	PT, aPTT	20 - 30 %	PCC
V	hemophilic type	PT a aPTT	15 - 20 %	FFP
VII	<1% hemophilic type	PT	15 - 20 %	FVII, PCC
VIII	joints, muscles	aPTT	40 - 50 %	FVIII
vWCH	mucous membrane, after trauma type 3 hemophilic type	aPTT, BT	40 - 50 %	vWF/FVIII
IX	Joints, muscles	aPTT	40 - 50 %	FIX
X	<1% hemophilic type	PT, aPTT	15 - 20 %	PCC
XI	Obstetric, dental procedures	aPTT	30 – 45 %	FFP, (FXI)
XII	**no bleeding**	aPTT		**no need of treatment**
XIII	Umbilical , CNS, soft tissues	fibrinolysis	3 - 5 %	FXIII, FFP
PK	**no bleeding**	aPTT		**no need of treatment**
HMWK	**No bleeding**	aPTT		**no need of treatment**
a2AP	hemophilic type, to bones	fibrinolysis	?	antifibrinolytics, FFP
PAI-1	after trauma,surgry	fibrinolysis	?	antifibrinolytics, FFP

Combined defects

Type:

- **I FV+VIII**
- **II FVIII+IX**
- **III FII+VII+IX+X, PC, PS**
  - **(vitamin K dependent factors)**
- **IV FVII+VIII**
- **V FVIII+IX+XI**
- **VI FIX+XI**

aPTT – etiology of prolongation

- **deficit:**
  - **FVIII, FIX, FXI, FXII**
  - FII, FV, FX
- **lupus anticoagulans**
- **heparin (↑ ↑ TT, normal reptilase time)**
- **dabigatran (↑ ↑ ↑ TT)**
- **acquired inhibitor (mostly against FVIII)**
- severe hypofibrinogenemia
- ↑ PCV (packed cell volume)

PT – etiology of prolongation

- **deficit:**
  - **FVII**
  - FII, FV, FX
- **Warfarin therapy**
- **Xabans – only mild increase inconstantly**
- lupus anticoagulans
- severe hypofibrinogenemia
- very seldom acquired inhibitor (FVII)
- ↑ PCV (packed cell volume)

Procoagulation factors in new-borns

Stable level:

- **fibrinogen > 1,5 g / l**
- **FVIII > 50%**
- **FV, FXIII > 30 – 40%**

Low level:

- **FII, FVII > 25% ∅40-60%**
- **FIX, FX, FXI, FXII > 10-15% ∅30-50%**
  - **½ year – 18 years > 50% (level is 80-85% as in adults)**
  - **In adults > 50 -60%**

Higher level:

- **VWF – during the first 3 months**

Koagulogram of new-born baby

- PT d1 d90 d180
  < 1,6 INR < 1,26 INR < 1,2 INR
- aPTT
  < 1,6 R < 1,5 R < 1,28 R

Coagulation inhibitors in new-borns

Lower level:

- AT III > 40% (∅60%) normalization (> 80%) in month 4-6
  > 15% (∅40%) if born in 30 – 36 gestation week
- PC > 15% (∅35%)
  > 30% month 3 > 40% 1/2 – 5 years
  > 45% 5 – 10 year > 55% - adult
- PS > 15% (∅35%) > 55% after month 3
- HCII > 10% (∅45%) > 50% after month 6

Elevation:

- α2MG > 100% normalization in 20 years

Influence on coagulation factors:

Gestation:

↑ Fbg, FVII, FVIII, VWF, FIX, FX, FXII, PAI

↓ PS

OC:

↑ Fbg, FVII, FVIII, VWF, FIX, FXII, FII, X, XI

↓ PS, AT III

Inflamation:

↑ Fbg, FV, FVII, FVIII, VWF

↑ α1AT, PAI, tPA, α2MG, Plg

Stress:

↑ Fbg, FVII, FVIII, VWF

↑ tPA

↓α2AP, Plg