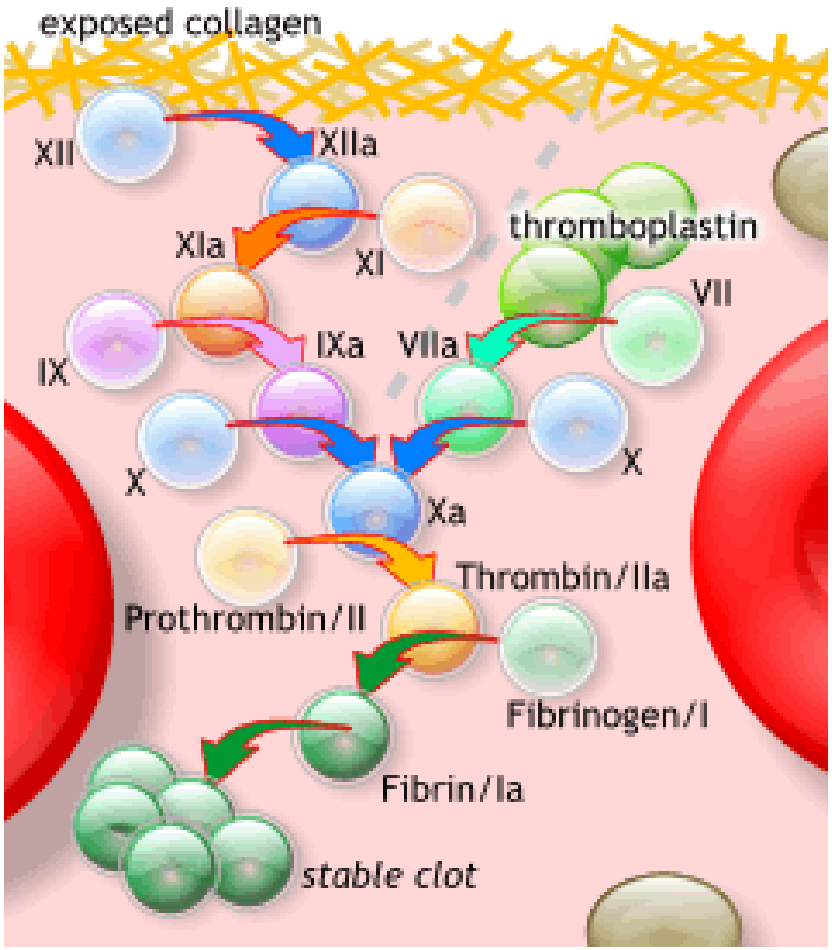
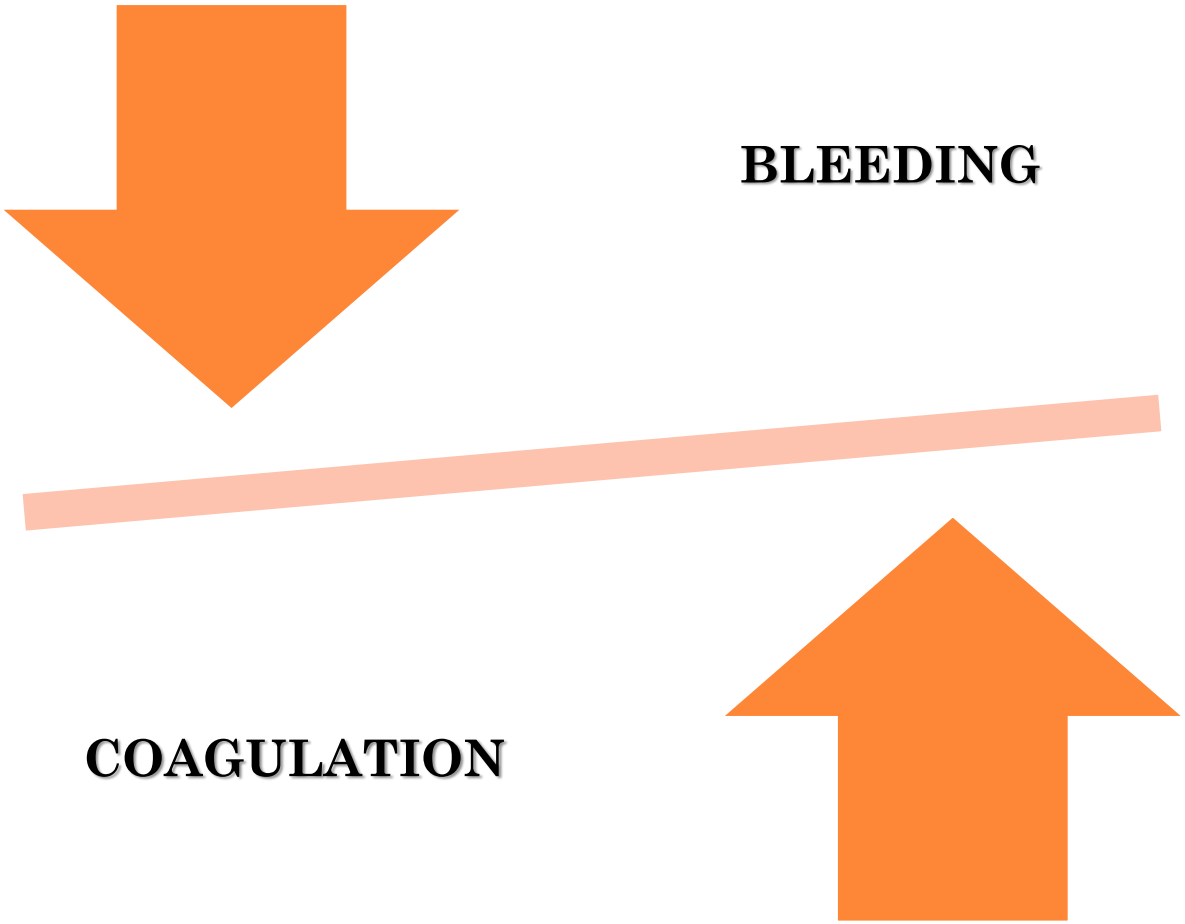


HAEMOSTASIS

10.10.2019

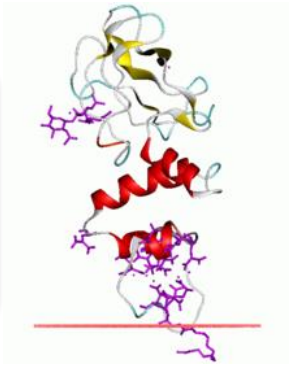
Gabriella Kiss

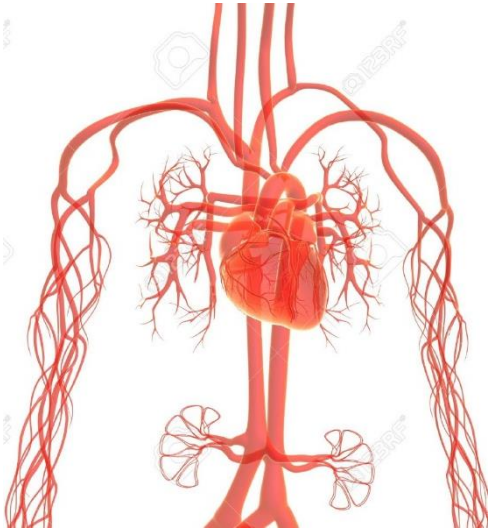
HAEMOSTASIS SYSTEM

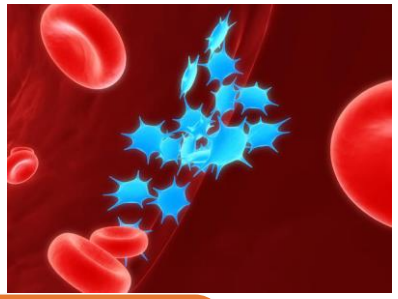


THE PARTICIPANTS

HUMORAL SYSTEM







VASCULAR SYSTEM

CELLULAR SYSTEM

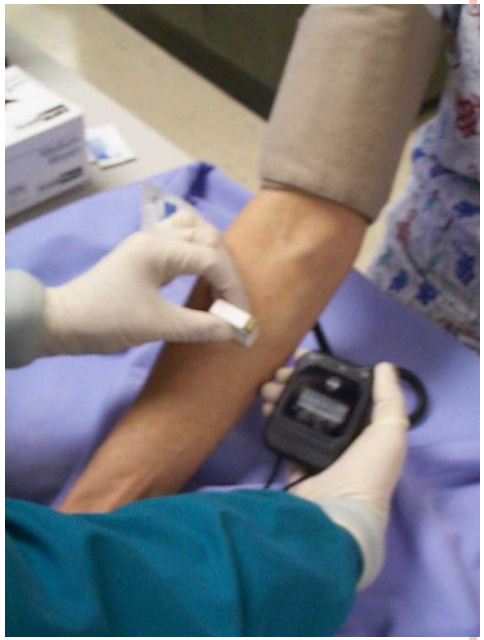
PREANALYTICS

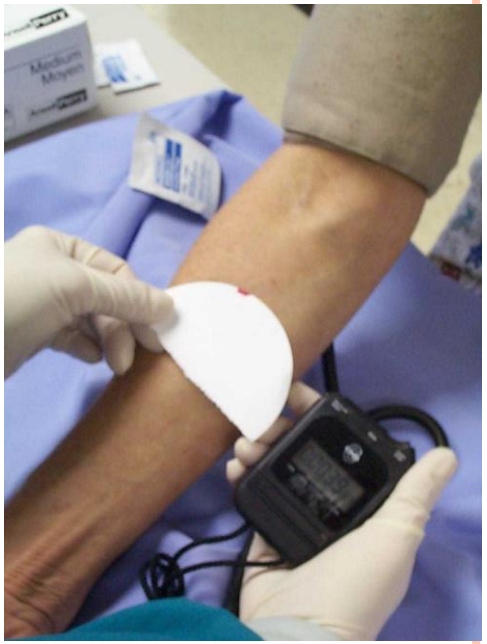
- anticoagulant additive: sodium citrate (blue cap tube)
- ratio of trisodium-citrate:blood= 1:9
- first tube when sampling
  - patient preparation, posture
  - avoid stasis
  - do not take sample from canules!!!
  - filling of tube (min. 90%)
  - do not shake, mix it gently

- sources of errors: hemolysis, lipaemia, heparin, clots
- transportation: 15-20 °C, prevent shaking
- must be measured within 2 hours
- sample preparation with different centrifugation: PPP, PRP

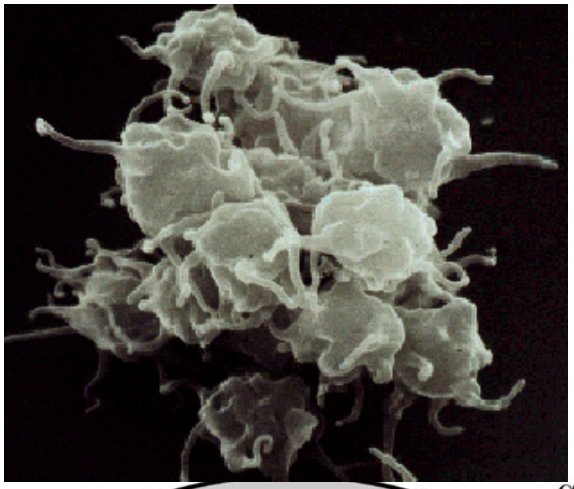
Global tests

- to determine bleeding time: modified Ivy method
- inflate blood pressure cuff: 40 Hgmm,
  - standard incision on ventral side of forearm (1mm)
  - every 30 seconds, a filter paper is used to blot the blood
  - reference range: 3-10 min.
  - information about platelets and vascular function
- clotting time
  - reference value: 2-6 min.

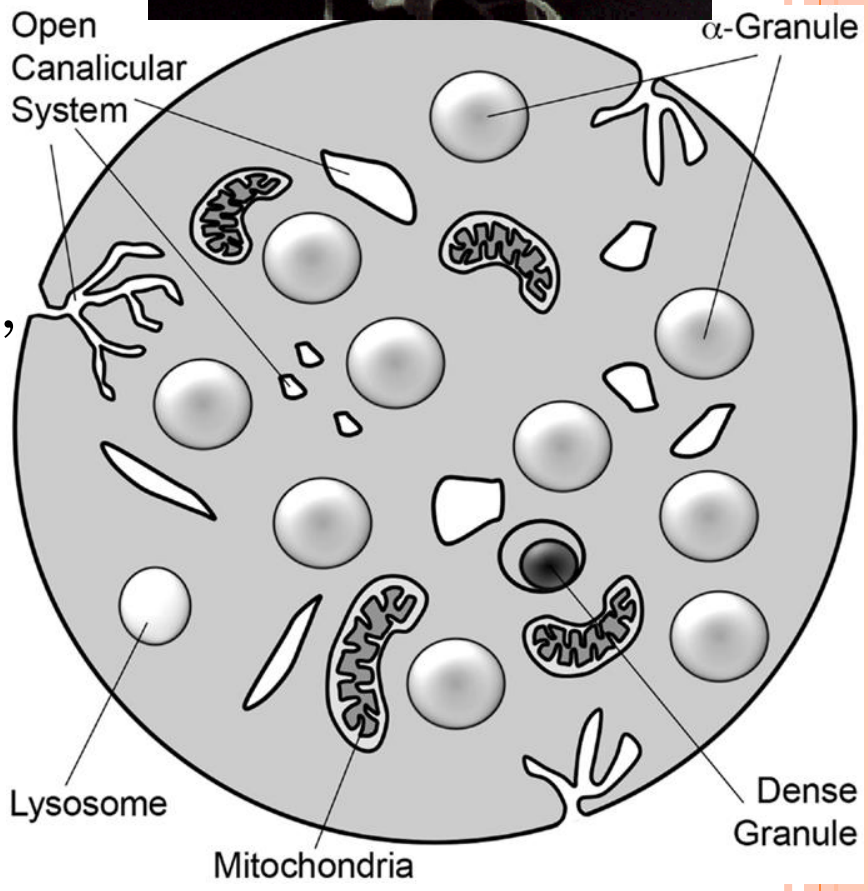




PLATELETS 1.

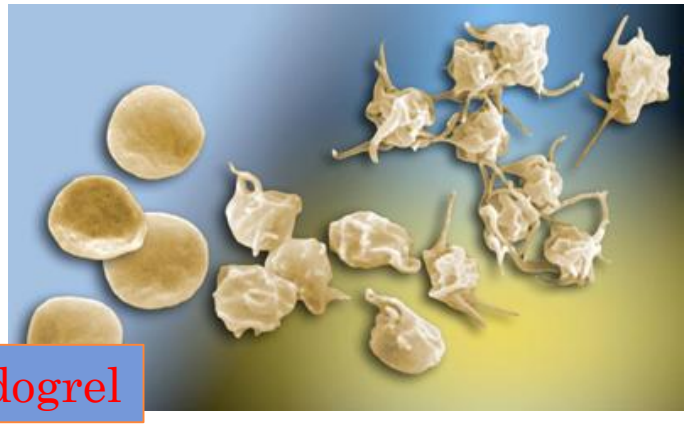


1. £-granules:
   - •growth/stimualting factors: IGF-1, PDGF, TGF-ß, PF4
   - •proteins taking part in coagulation: thrombospondin, fibronectin, vWF
   - •coagulation factors: fibrinogen, V, factor XIII
   - •others: P-selectin, CD63
2. dense-granules:
   - •ATP, ADP,
   - •Ca2+,
   - •histamine,
   - •serotonin



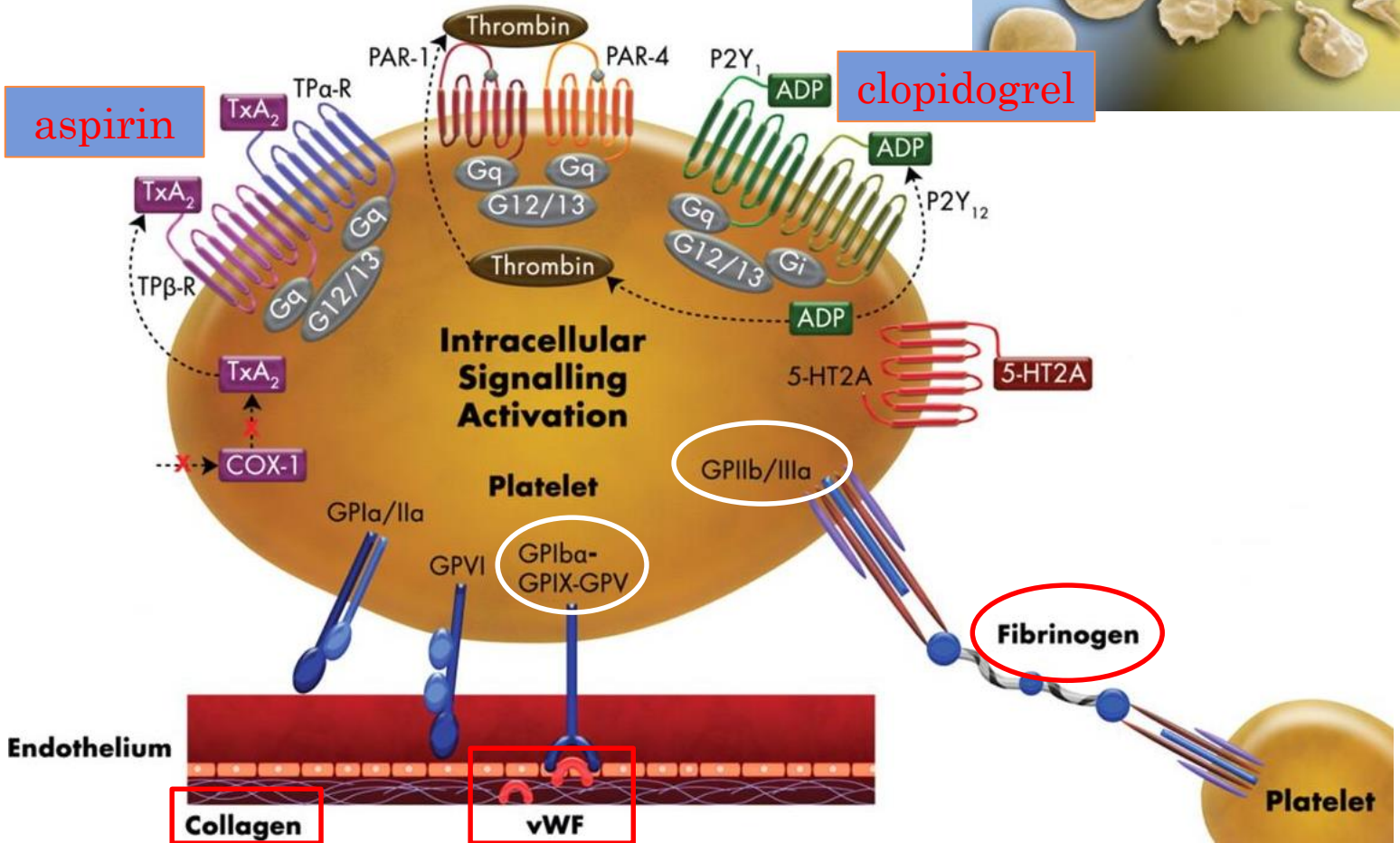
THROMBOCYTES 2.: AGGREGATION

1. adhesion (GPIb-vWF; GP Ia/IIa-collagen)
2. activation-release (through different receptors)
3. aggregation (GPIIb-IIIa-fibrin)



aspirin

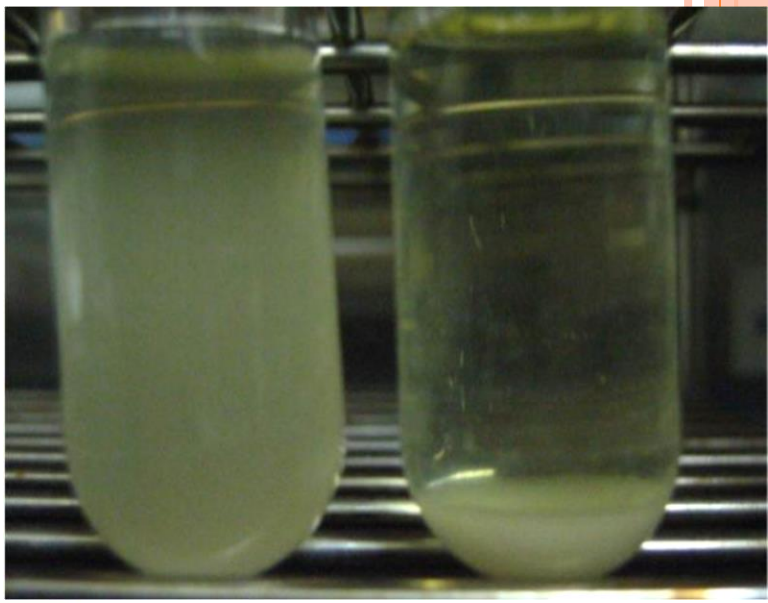
clopidogrel



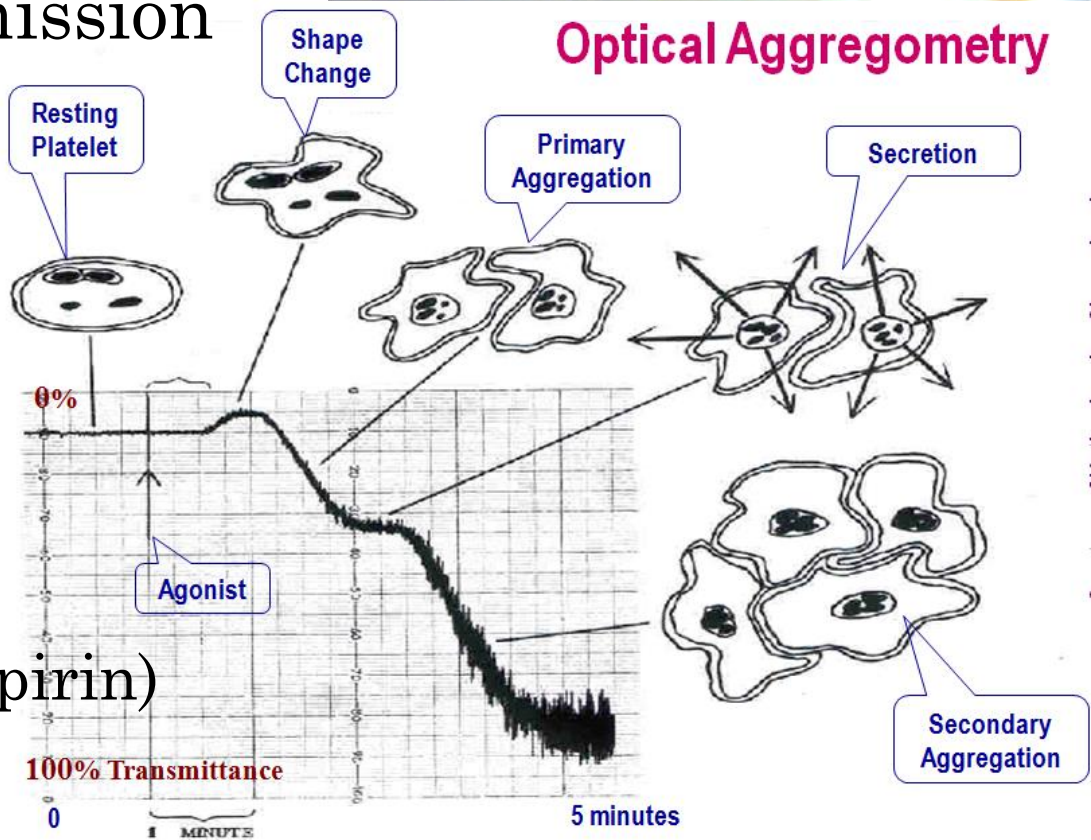
THROMBOCYTE EXAMINATIONS

- plt count
- life span determination (monitoring plt transfusion, radioactive labeling)
- function:
  - aggregometry (optical, impedance)
  - PFA-100 (determination of closing time)
  - lumi-aggregometry (examining ATP release)
  - flow cytometrical analysis of thrombotic markers (CD62P, PAC-1)

AGGREGOMETRY: OPTICAL



- PPP: 100% light transmission
- PRP: 0% light transmission
- agonists
  - ADP, (Clopidogrel)
  - adrenalin,
  - collagen,
  - thrombin,
  - ristocetin, (vWF)
  - arachidonic acid (Aspirin)



PÉCSI TUDOMÁNYEGYETEM
Klinikai Központ
Laboratóriumi Medicina Intézet
a NAT által NAT-1-1553/2012 számon
akkreditált vizsgálólaboratórium
Igazgató: Prof.dr. Miseta Attila egyetemi tanár
LMI Központi laboratórium
7624 Pécs Ifjúság útja 13 Tel: 72/535-823,32123 Fax: 72/536-121,20983

L E L E T

Thrombocyta aggregációs panel

Beteg neve.....: [redacted]
Születési dátum...: 1934.11.12
Anyja neve.....: Magassy Ida
Lakcím.....: 7691 Pécs-Vasas, Bencze József utca 2.
Vizsgálatkérő.int.: URF1 024211114 11114 Urológiai Kl. Általános+ito
Vizsgálatkérő.orv.:
47130 Fábos Zoltán Dr.
Iránydiagnózis....: D4110
Minta visszaigazolás: 2015.09.28 12:03

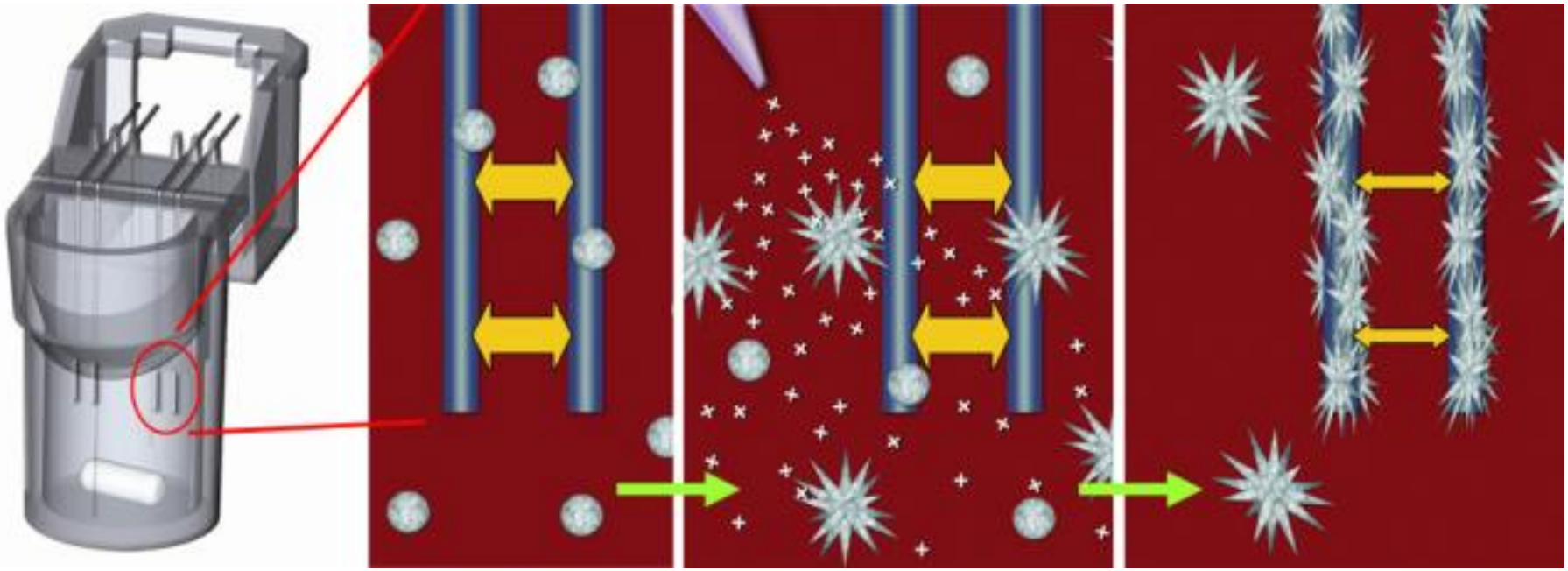
KBA.....: 00000402436
Esetszám: 10062125
Telj. AZ: 9936984
TAJ.....: 101-836-137

Megnevezés	Érték	Abn	Egység	Referencia tart.
ADP (5 uM) #	67	L	%	70-100
Arachidonsav - 0,5mg/ml	70		%	70-100
Terápia: Aspirin				
Ristocetin (1 mg/ml) #	68	L	%	70-100
Ristocetin (0,5 mg/ml) #	0		%	<5
Spontán #	0		%	<5
Terápia: Aspirin				

Ellenőrizte: X
Tőkés-Füzesi Margit dr. 2015.09.28 14:21

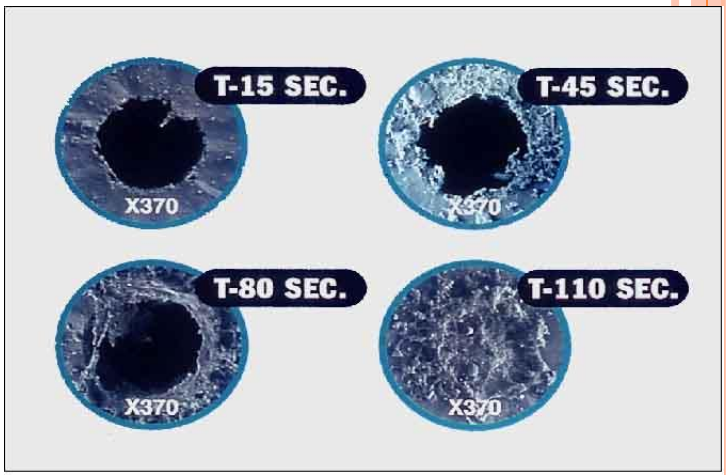
AGGREGOMETRY: IMPEDANCE

- Multiple Electrode Aggregometry
- measures the electric resistance changes between the two electrodes, when platelets aggregate in the surface of the electrodes



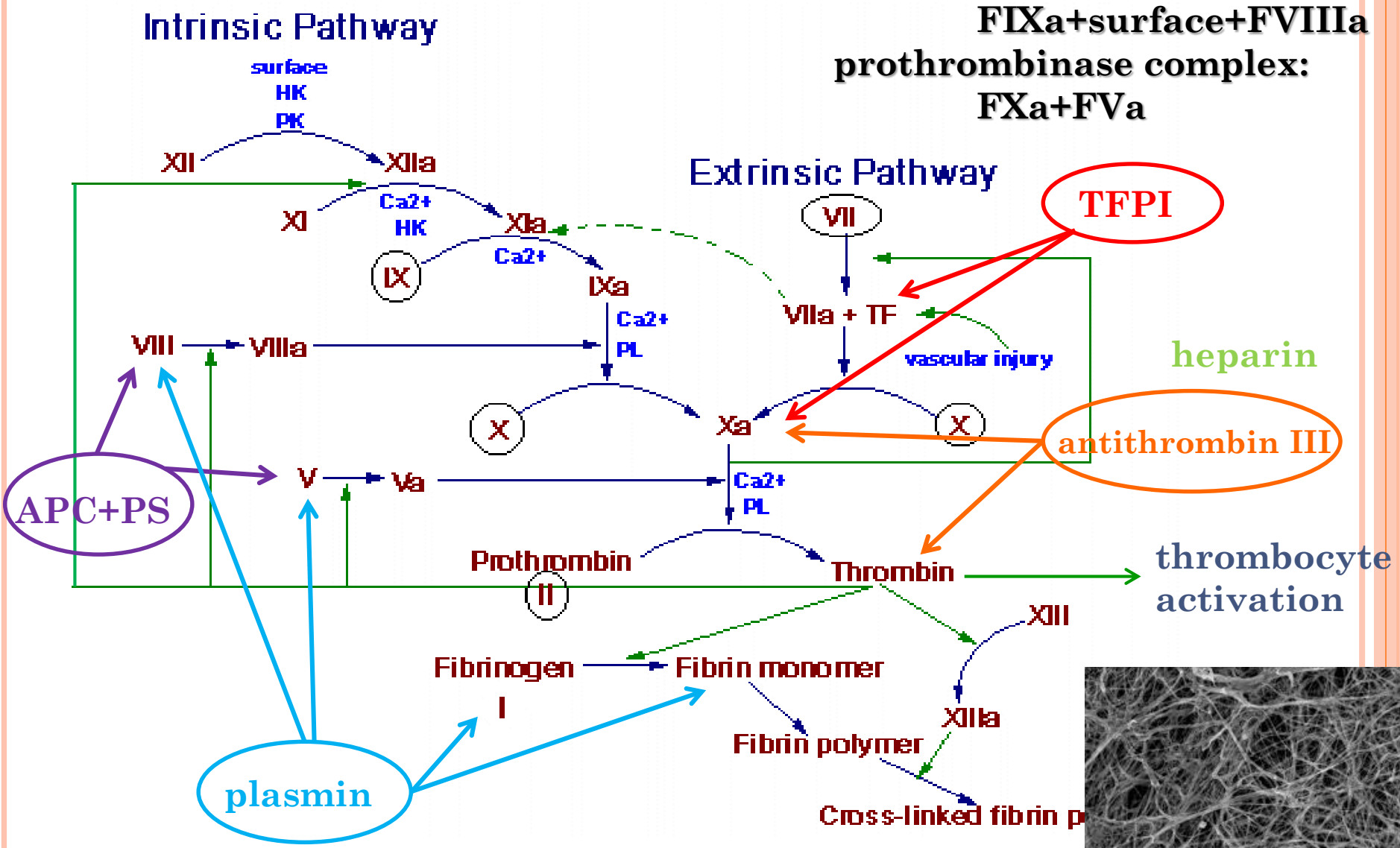
PFA-100 (PLT FUNCTION ANALYSER)

- a membrane impregnated with collagen and one plt activating factor (adrenalin, or ADP), with a hole in it (147 μm)
- anticoagulated whole blood passes through the membrane, simulating the shear stress developing in the capillaries in vivo
- platelets adhere to the margin of the hole, closing it after a while= CT-closing time
- ! also depends on the haematocrit and platelet count!



THE HUMORAL SYSTEM

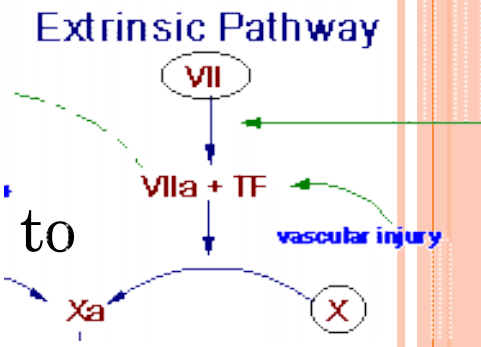
extrinsic tenase complex:
TF+FVIIa
intrinsic tenase complex:
FIXa+surface+FVIIIa
prothrombinase complex:
FXa+FVa



COAGULOMETERS

- measuring the time from adding the start reagent to clot formation
- photometric:
  - fibrin polymer: light absorbance at 405nm and 660nm
  - interfering factors: lipoprotein, bilirubin
  - difficult to find clotting end point (diff. methods)
- nephelometric:
  - light scattering depends on the size of the molecule: fibrinogen, fibrin can be easily discriminated

COAGULATION TESTS 1.: PROTHROMBIN TIME



- informs about the extrinsic pathway
- reagent: thromboplastin and calcium
- measuring the time from adding the reagent to clot formation (sec., or %)
- PR= patient PT/PT measured from normal reference plasma
- ref.range: PT: 80-120%; (INR: 0,9-1,15); PR: 0,85-1,15
- can be increased: vit. K deficiency (syncumar), liver disease, disturbance in extrinsic pathway factors, antiphospholipid antibody, increased ratio of citrate:blood
- suitable for monitoring oral anticoagulant therapy

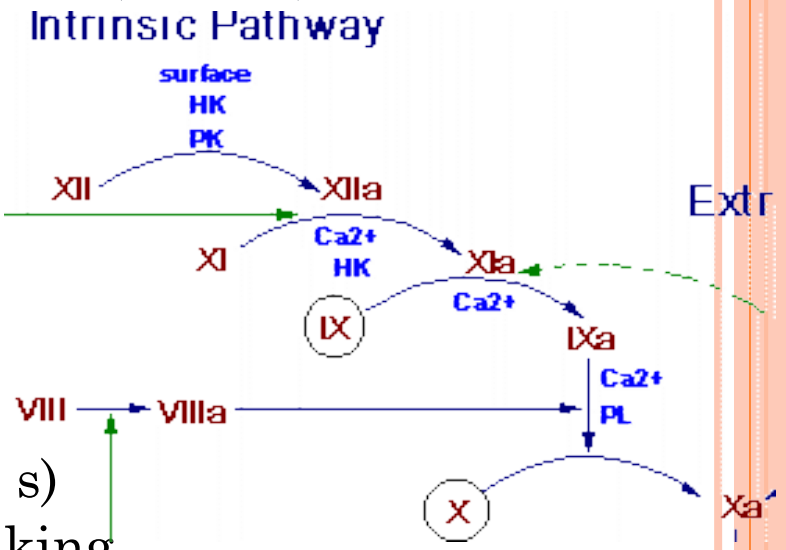
INR=International Normalized Ratio

- thromboplastin products made by various manufacturers have different efficiency: necessity of standardisation to ensure comparability of the results
- every manufactured thromboplastin has an ISI (= International Sensitivity Index) value= the efficiency correlated to the reference thromboplastin (ISI=1,0)

$$INR = PR^{ISI}$$

- to monitor anticoagulant therapy (syncumar)
- reference ranges are different, depending on the purpose of the therapy (profilaxys for venous thrombosis: 2-3, pulmonary embolism: 2-4, mechanical valve: 3-4,5)

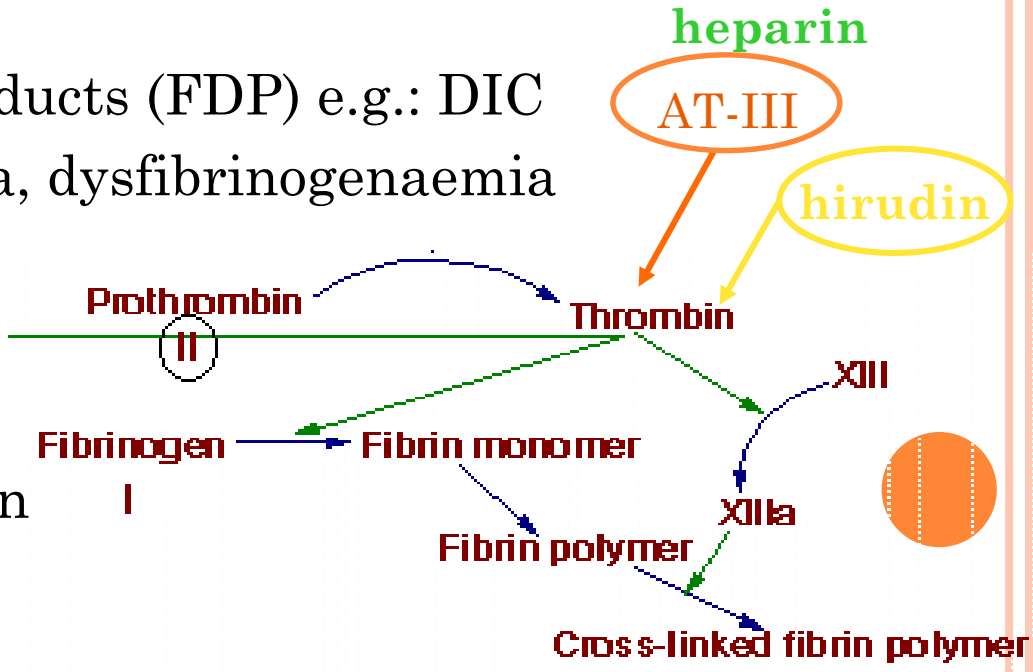
COAGULATION TESTS 2.: ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT)



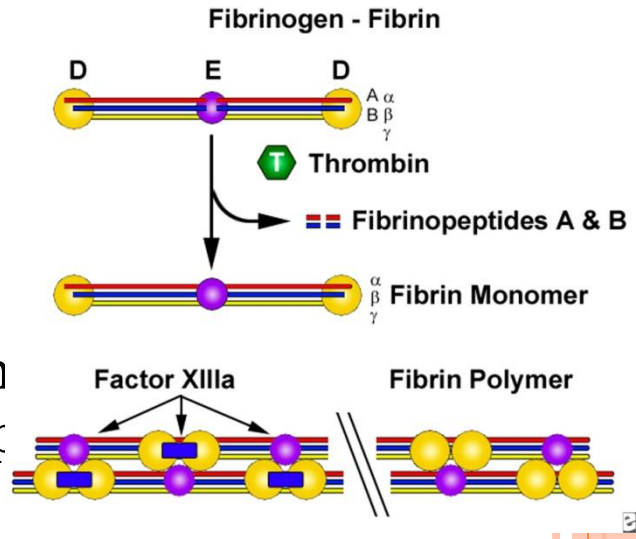
- informs about the <u>intrinsic pathway</u>
- start reagent: contact activator ensuring negative surface (pl. kaolinite, or silica), phospholipid (partial thromboplastin) and calcium
- measuring the time from adding the start reagent to clot formation (ref. range: 27-33 s)
- information about all of the components taking part in coagulation except for factor VII
- suitable for:
  - <u>monitoring heparin th.</u> (unfractionated)
  - screening hereditary/acquired coagulopathies
  - presence of inhibitors
  - presence of lupus anticoagulant
  - diagnosing acute DIC
  - following thrombolytic therapy
  - monitoring oral anticoagulant therapy
  - diagnosing dysfibrinogenemias

COAGULATION TESTS 3.: THROMBIN TIME (TT)

- informs about <u>fibrin polymerisation</u>
- reagent: determined quantity of thrombin
- measuring the time from adding thrombin to clot formation (ref. range: 18-25 s)
- prolonged TT:
  - fibrin degradation products (FDP) e.g.: DIC
  - hypo-afibrinogenaemia, dysfibrinogenaemia
  - heparin th.
  - liver desease
  - hirudin th.
  - presence of paraprotein



ANALYSING FACTORS:
DETERMINATION OF FIBRINOGEN

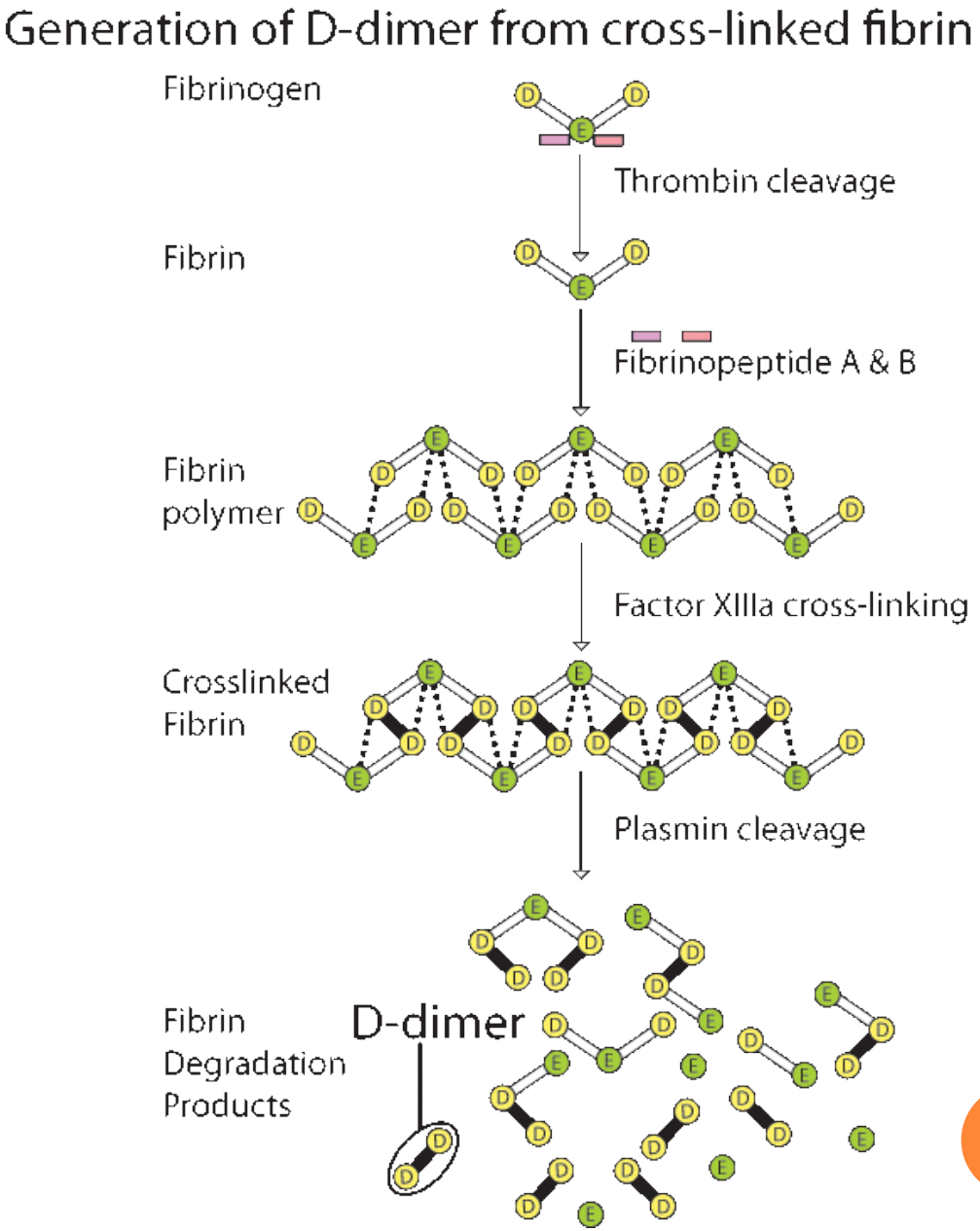


Clauss-method:

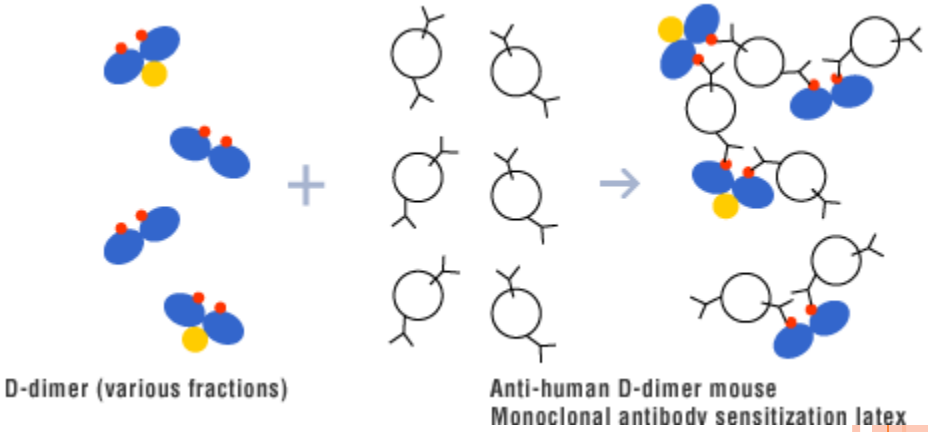
- in the presence of high concentration of th
  clotting time of diluted plasma mainly dep
  fibrinogen concentration of it
- calibration curve with calibrator (known fibrinogen concentration)
- !heparin moderates it
- ref. range: 2-4 g/l

- immune turbidimetry/immunonephelometry:
  - non functional
  - cannot exclude FDP (it reacts with FDP-s)
- concentration decrease.: DIC, liver disease
- concentration increase: acute phase reaction, chronic inflammation, nephrosis-sy., inherited hyperfibrinogenaemia

D-DIMER


Generation of D-dimer from cross-linked fibrin
Fibrinogen
Thrombin cleavage
Fibrin
Fibrinopeptide A & B
Fibrin polymer
Factor XIIIa cross-linking
Crosslinked Fibrin
Plasmin cleavage
Fibrin Degradation Products
D-dimer

D-DIMER



- determination:
  - rapid tests (agglutination)
  - turbidimetry:
    - latex beads coated with monoclonal antibodies
    - in the presence of D-dimer aggregation occurs: turbidity decreases
    - calibration curve: with standard of known concentration
  - fluorescent immunoassay
  - ELISA
- informational value:
  - elevated over the age 70, and in pregnancy
  - DIC, pulmonal embolism, deep venous thrombosis, tumor, severe infection
  - thrombolysis
  - negative predictive value: 100%

CONTROLLING ANTICOAGULANT THERAPY

- unfractionated heparin: APTT (required: 1,5-3x)
- LMWH: unneeded, except for: unexpected bleeding, kidney failure patients, in the case of DVT treatment. Test for it: factor Xa inhibition test
- low dose prophilactic LMWH: unneded
- oral anticoagulant therapy: PT
- conversion therapy from heparin to oral anticoagulant: PT, APTT (36-48 hours after conversion, then every other day: PT)
- during fibrinolytic th.: TT (before beginning it: screen for hemorrhagic diathesis)

DABIGATRAN

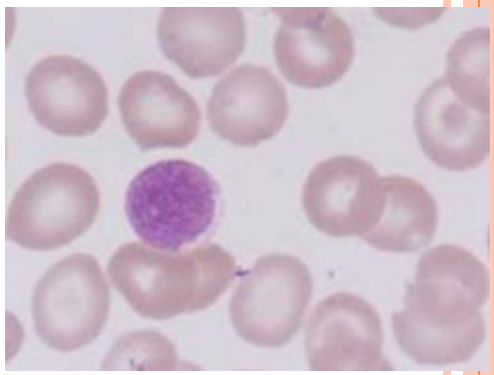
RIVAROXABAN

HAEMOSTASIS DEFECTS: SUMMARY

1. hemorrhagic diathesises:
   - 1.1 thrombopathies (inherited/ acquired)
   - 1.2 coagulopathies (inherited/ acquired)
   - 1.3 vasculopathies
2. thrombotic states
3. thrombophilias
   - 3.1 inherited
   - 3.2 acquired

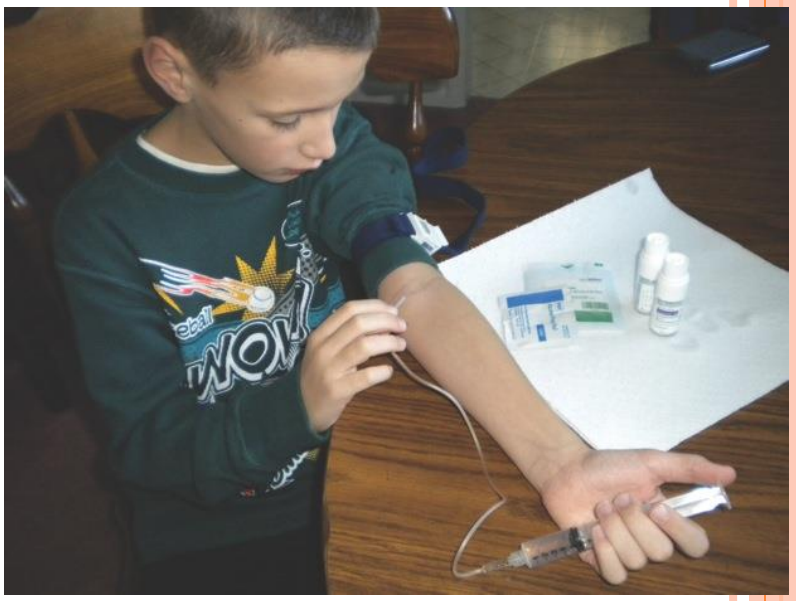
THROMBOPATHIES

- plt count, bleeding time, aggregometry (examine aggregation and secretion), PFA-100 (adhesion-aggregation), flow cytometry (examining plt receptors)
- the most common inherited diseases:
  - von Willebrand disease, Bernard Soulier-sy. (adhesion)
  - Glanzmann-thrombasthenia (aggregation)
  - storage pool deficiencies (secretion)
- acquired disorders:
  - medications
  - uraemia
  - haematologic diseases (associated with myeloproliferative diseases)
  - associated with liver diseases

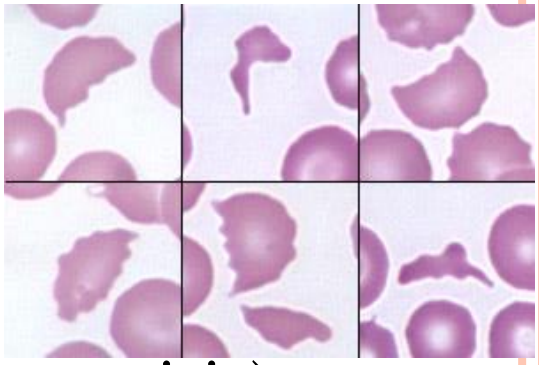


COAGULOPATHIES 1.

- inherited: decreased function or deficiency of factors (eg. hemophilia A)
- acquired:
  - liver disease
  - consumption (DIC)
  - fibrinolytic therapy
  - inhibitors
- procedure of investigation:
  - screening test (PT, APTT, TT)
  - sample check (citrate ratio, heparin contamination)
  - special tests (mixing studies, determination of inhibitors, factor- analysis)



COAGULOPATHIES 2.: DIC



risk factors:

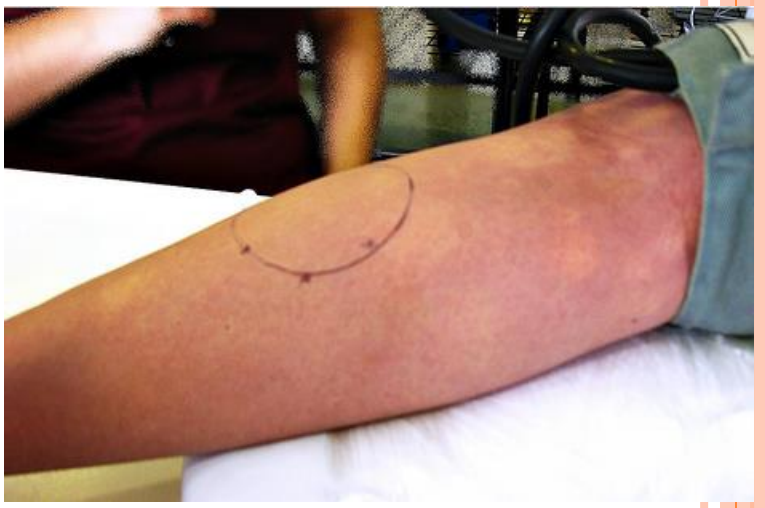
1. infection (sepsis)
2. trauma/tissue damage (head injury, pancreatitis)
3. malignant diseases (tumor, acute leukaemia, CMMoL)
4. pregnancy/delivery (preecclampsia/ecclampsia, dead fetus, abruptio placentae)
5. allergy/toxins (toxic shock, snake venom, acute post transfusion haemolytic reaction)

by the International Society of Thrombosis and Haemostasis (5)

Points	0	1	2	3
Platelet, count/nL	>100	≥50	<50	
D-dimer, μg/mL	≤1.0		1.0–5.0	>5.0
Fibrinogen, g/L	>1.0	≤1.0		
Prothrombin index, %	>70	40–70	<40	

The score ranges from 0 to 8 points. A scoring system for DIC of ≥5 points is compatible with overt

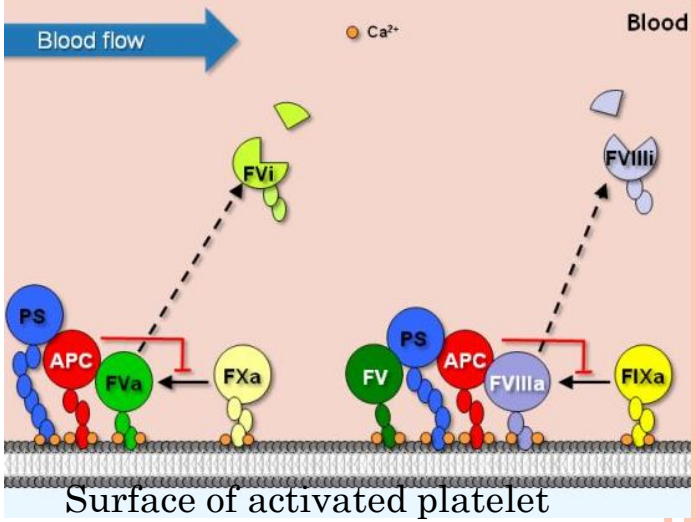
VASCULOPATHIES



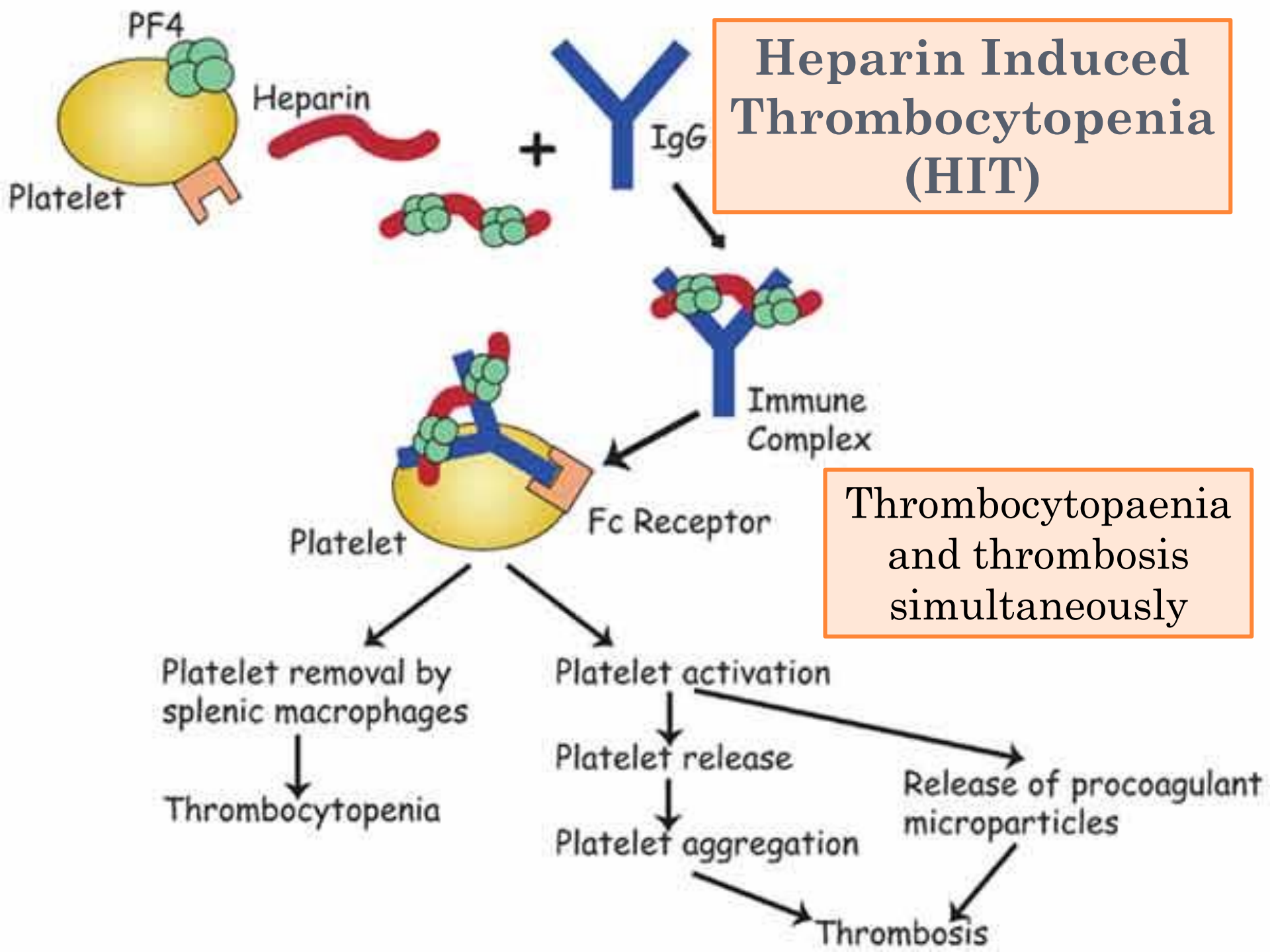
- diagnosis of exclusion
- prolonged bleeding time
- plt count and function is normal
- Rumpel-Leede-test (apply a cuff inflated to mean arterial pressure for 5 minutes, count the appearing petechiae)
- inherited: Ehlers-Danlos- sy., Marfan- sy.
- acquired: medicals

THROMBOPHILIAS

- inherited:
  - inhibitor deficiency (ATIII deficiency)
  - dysfunction of inactivating system (protein C and protein S, APC resistency: most common: Leiden-mutation)
  - increased factor levels
  - hyperhomocysteinaemia
- acquired:
  - anti phospholipid syndrome (APS)-lupus anticoagulant, or anti cardiolipin antibody
  - (HIT)



Heparin Induced Thrombocytopenia (HIT)



Thrombocytopaenia and thrombosis simultaneously

DIAGNOSTIC CRITERIA OF HIT

- **based on 4T score**

6-8 points:
high risk (hirudin, Dabigatran)
4-5 points:
middle risk
0-3 points:
low risk

Category	2 points	1 point	0 point
Thrombocytopenia	> 50% fall, or nadir ≥ 20 x 10^9/L	30–50% fall, or nadir 10-19 x 10^9/L	< 30% fall, or nadir < 10 x 10^9/L
Timing of the decrease in platelet count	Days 5 to 10, or ≤ day 1 with recent heparin (past 30 days)	> Day 10 or timing unclear, or < day 1 if heparin exposure within past 30-100 days	< Day 4 (no recent heparin)
Thrombosis or other sequelae	Proven thrombosis, skin necrosis, or acute systemic reaction after heparin bolus	Progressive, recurrent, or silent thrombosis; erythematous skin lesions	None
Other causes of thrombocytopenia	None evident	Possible	Definite

THANK YOU FOR YOUR ATTENTION!