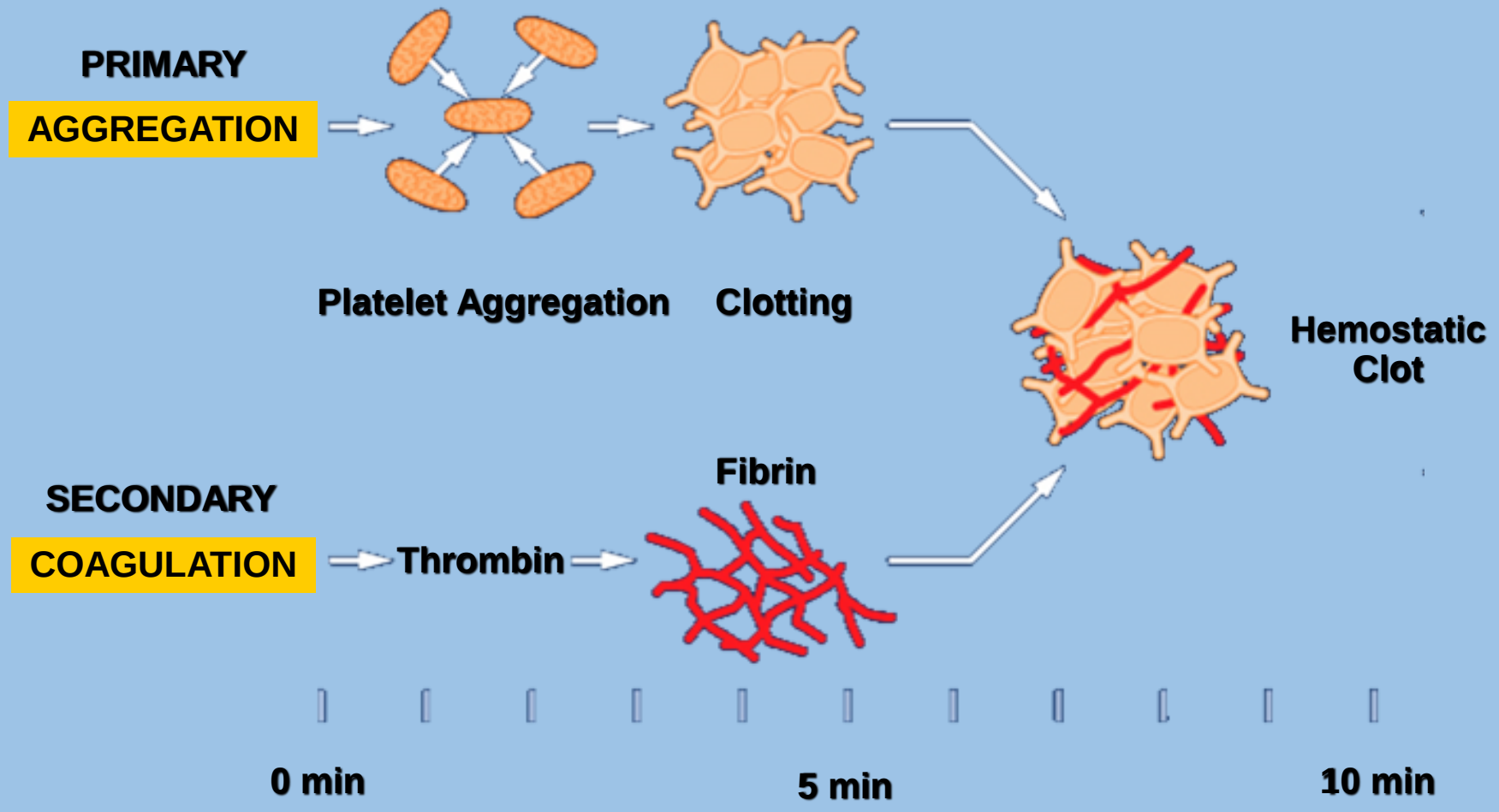
Primary haemostasis

Platelet physiology

- The main function of platelets is to contribute to hemostasis: the **process of stopping bleeding** at the site of vessel injuries
- First, platelets attach to substances outside the interrupted endothelium: ***adhesion***. Second, they change shape, turn on receptors and secrete chemical messengers: ***activation***. Third, they connect to each other through receptor bridges: ***aggregation***
- Formation of this platelet plug (primary hemostasis) is associated with activation of the coagulation cascade with resultant fibrin deposition and linking (secondary hemostasis).

Hemostatic Plug Formation



Adapted from Ferguson JJ, et al. *Antiplatelet Therapy in Clinical Practice*. 2000:15-35.

Platelets : 140 – 450 x 10^9 /l

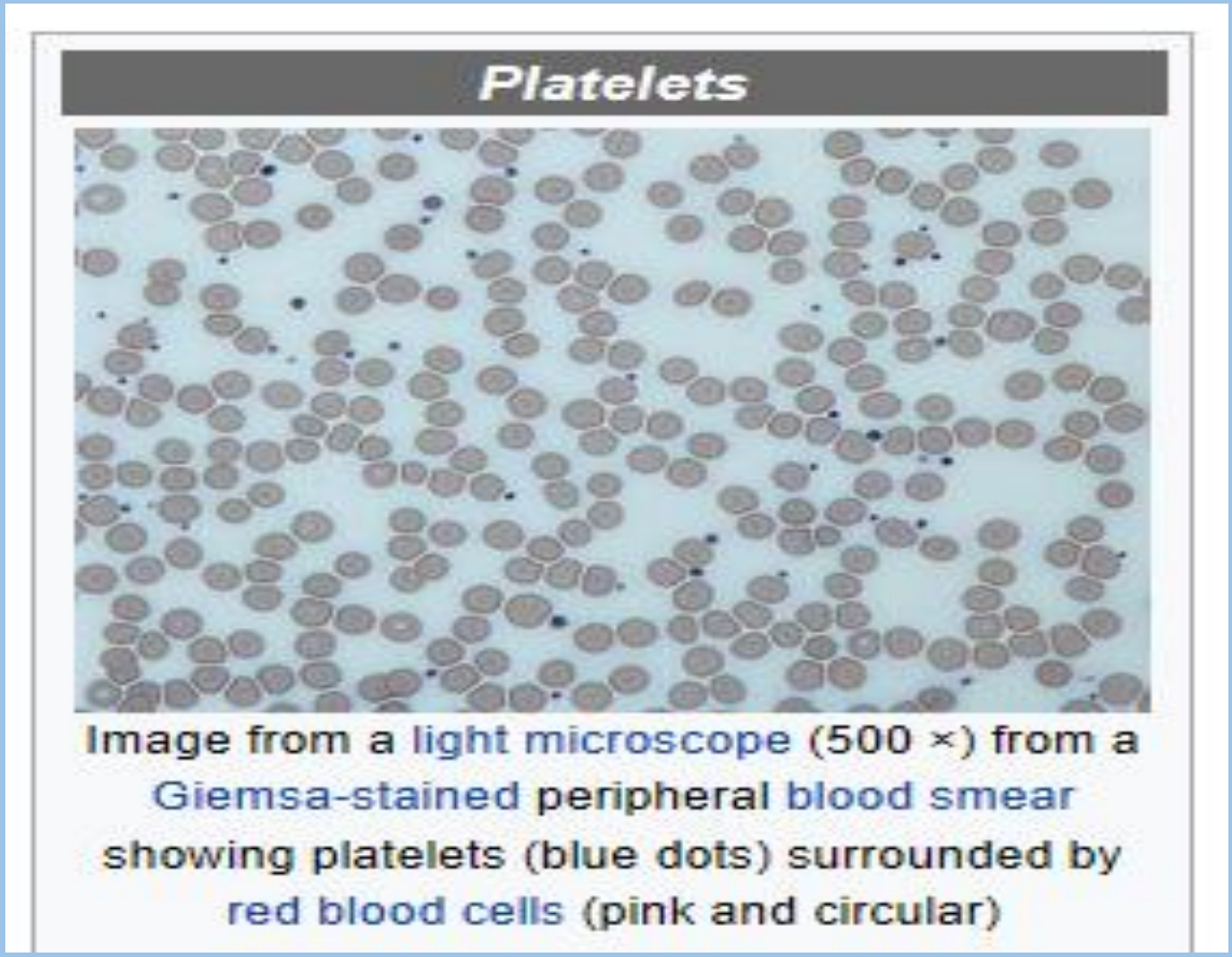
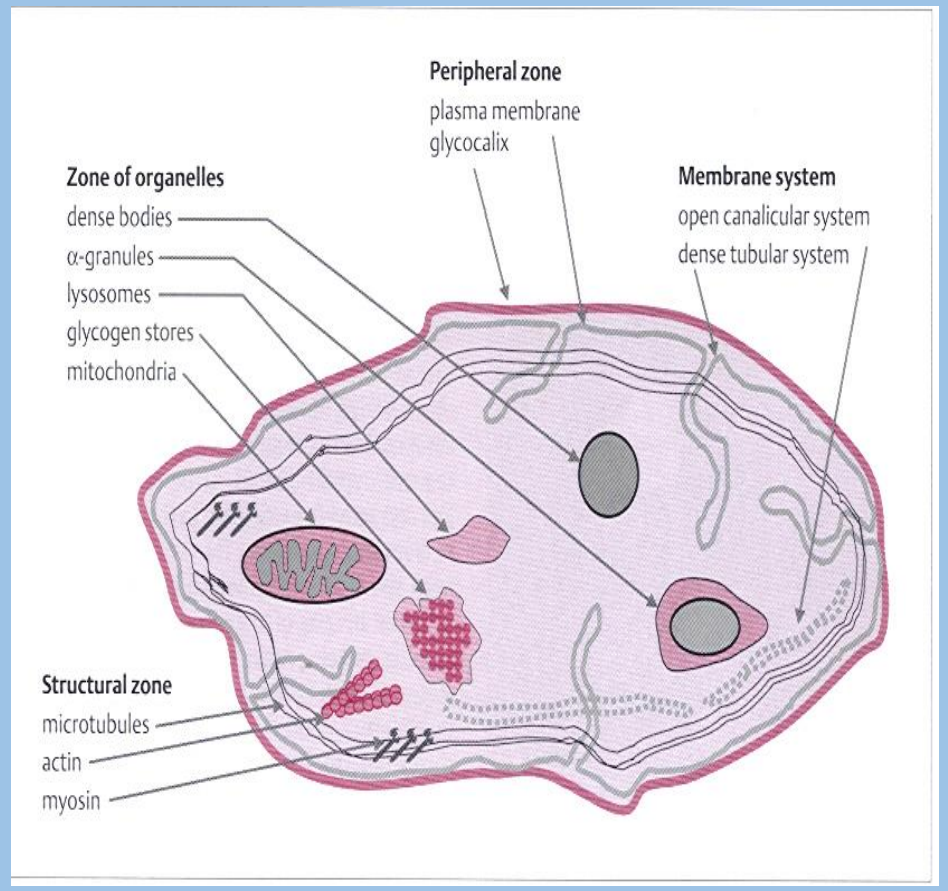


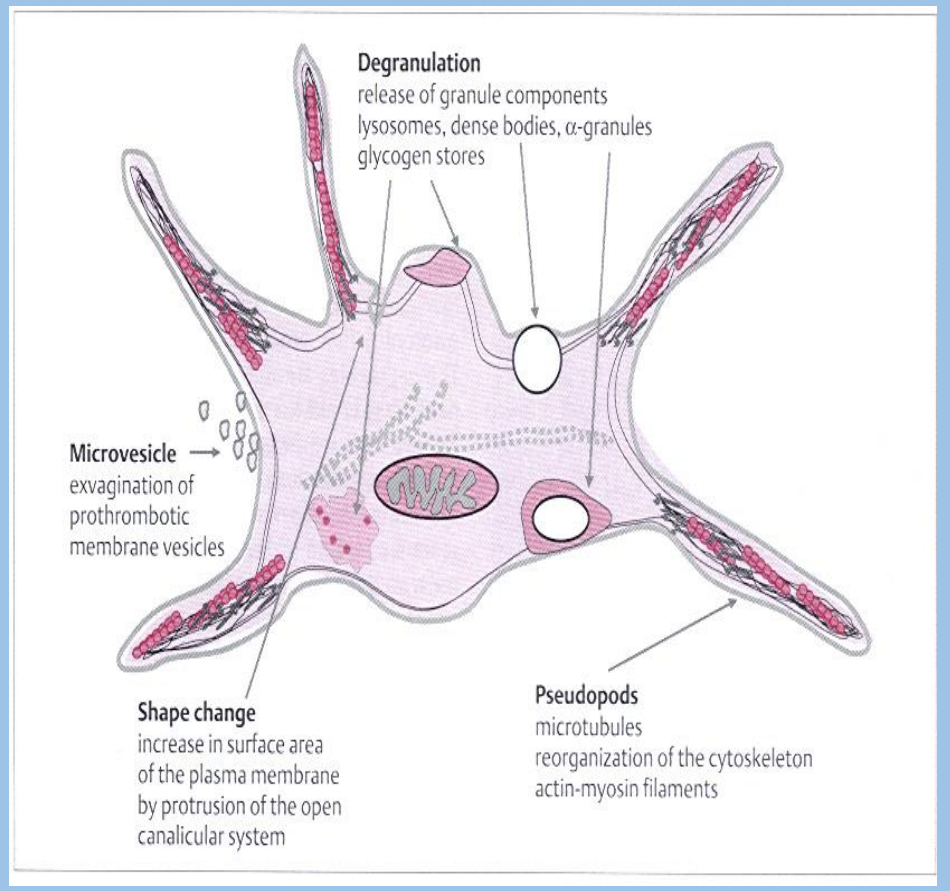
Image from a light microscope (500 ×) from a Giemsa-stained peripheral blood smear showing platelets (blue dots) surrounded by red blood cells (pink and circular)

Platelets

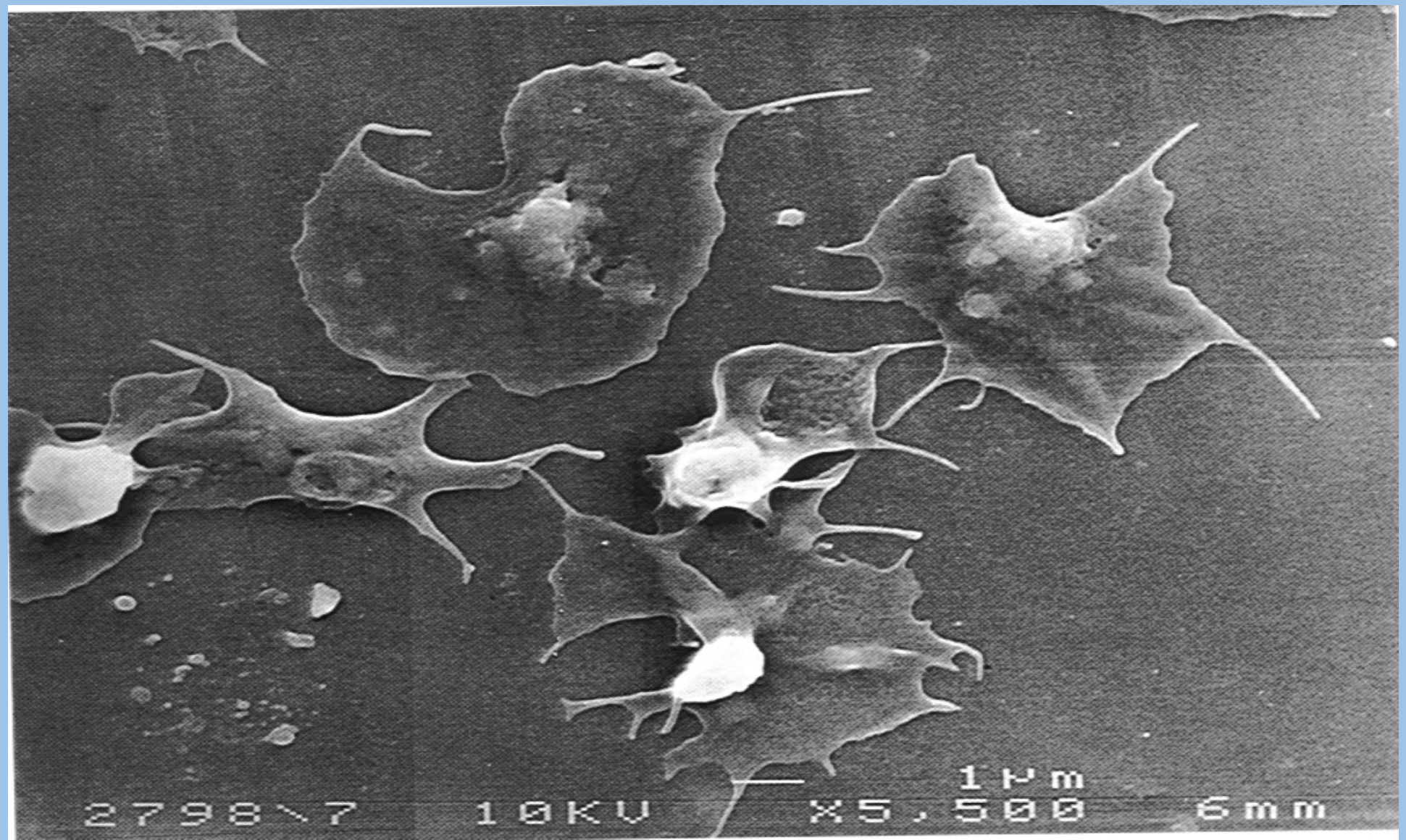
unactivated



activated



Platelet adhesion



Primary hemostasis

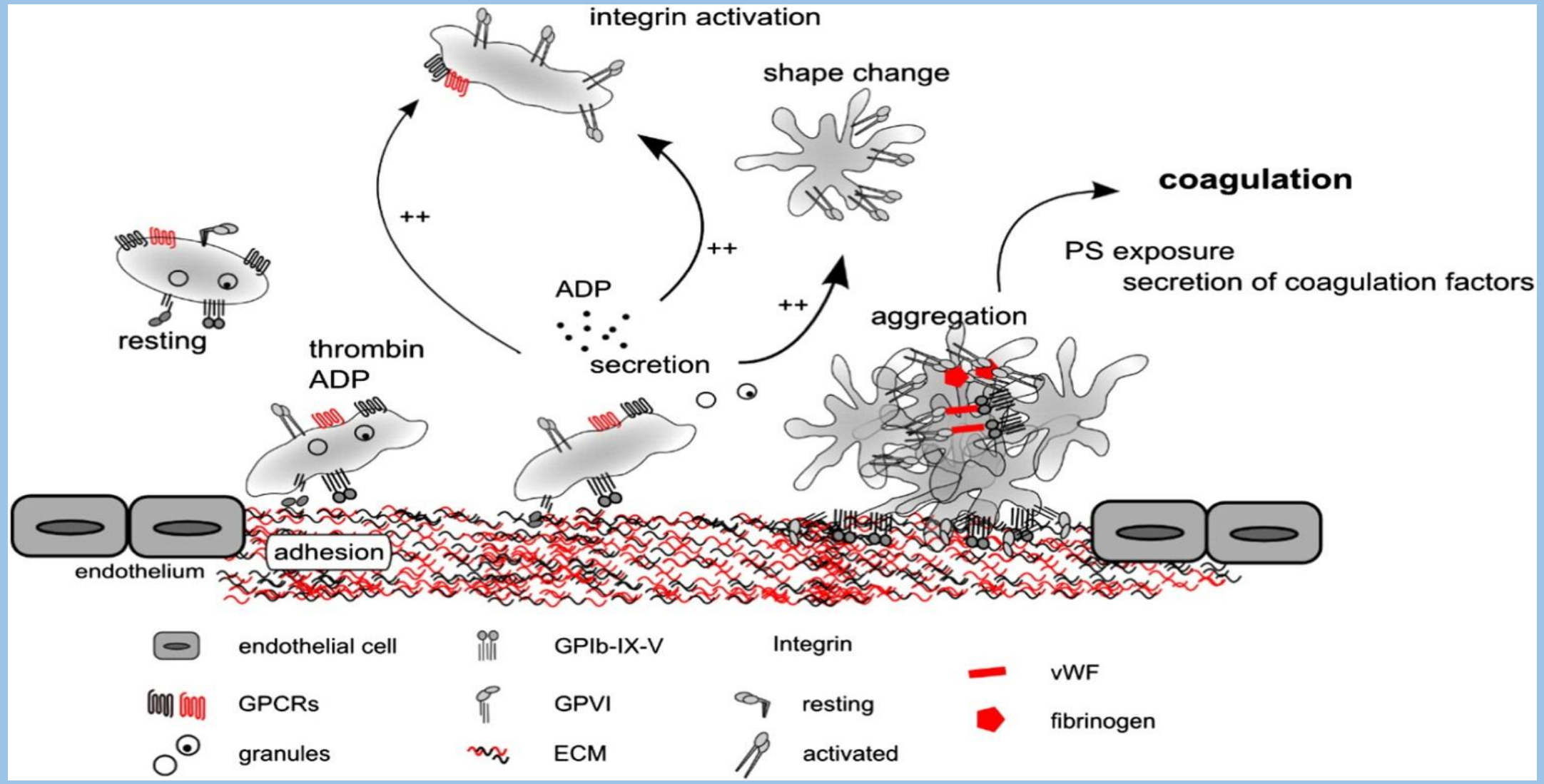
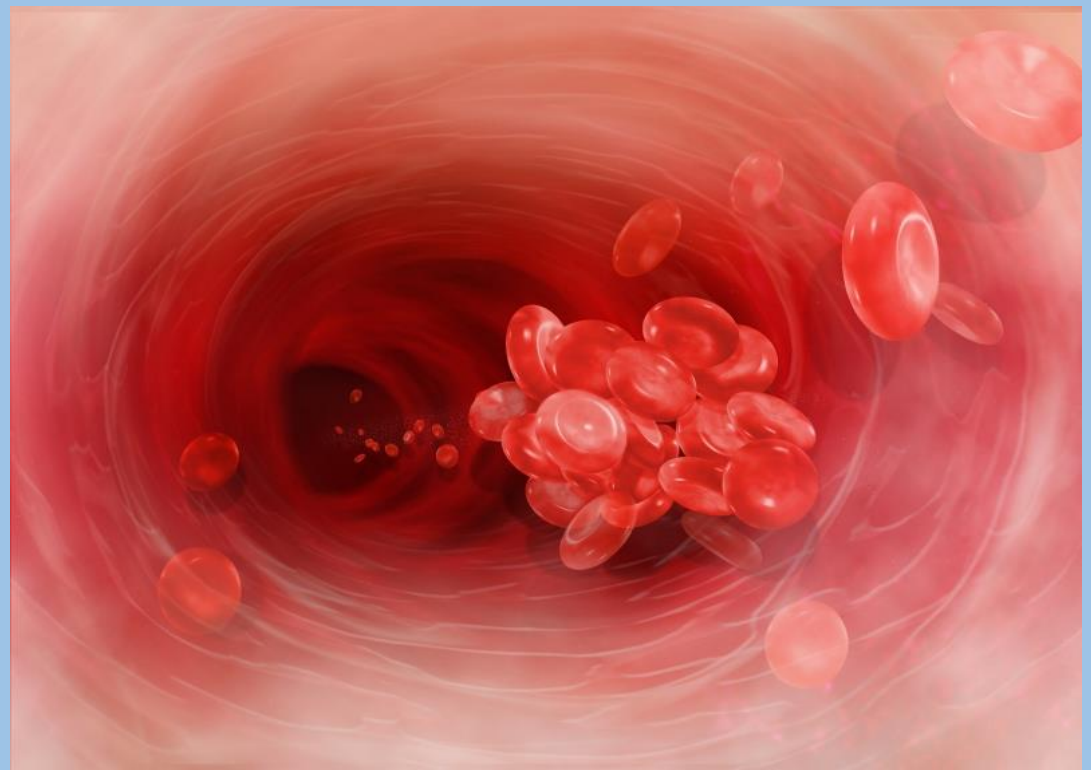


Table 1: Major platelet membrane receptors and their ligands

Glycoprotein (GP) Receptor	Structure	Function / Ligand
GP IIb/IIIa	Integrin $\alpha_{IIb}\beta_3$	Receptor for fibrinogen, VWF, fibronectin, vitronectin and thrombospondin
GP Ia/IIa	Integrin $\alpha 2\beta 1$	Receptor for collagen
GP Ib/IX/V	Leucine-rich repeats receptor	Receptor for insoluble VWF
GP VI	Non-integrin receptor, Immunoglobulin superfamily receptor	Receptor for collagen

Bleeding disorders due to primary hemostasis malfunction



Platelet pathophysiology

- Low platelet concentration is thrombocytopenia and is due to either *decreased production* or *increased destruction of platelets*.
- Elevated platelet concentration is thrombocytosis and is either *congenital*, *reactive* (to cytokines), or due to *unregulated production of platelets* at the *myeloproliferative neoplasms.*
- A disorder of platelet function is a thrombocytopathy

A) Thrombocytopenia

- Platelets count : < 140 x10^9/l

- thrombocytopenia is due to either :

- *A) decreased production* of platelets (ITP)

  or

- *B) increased destruction of platelets (in microangiopathy,TTP)*

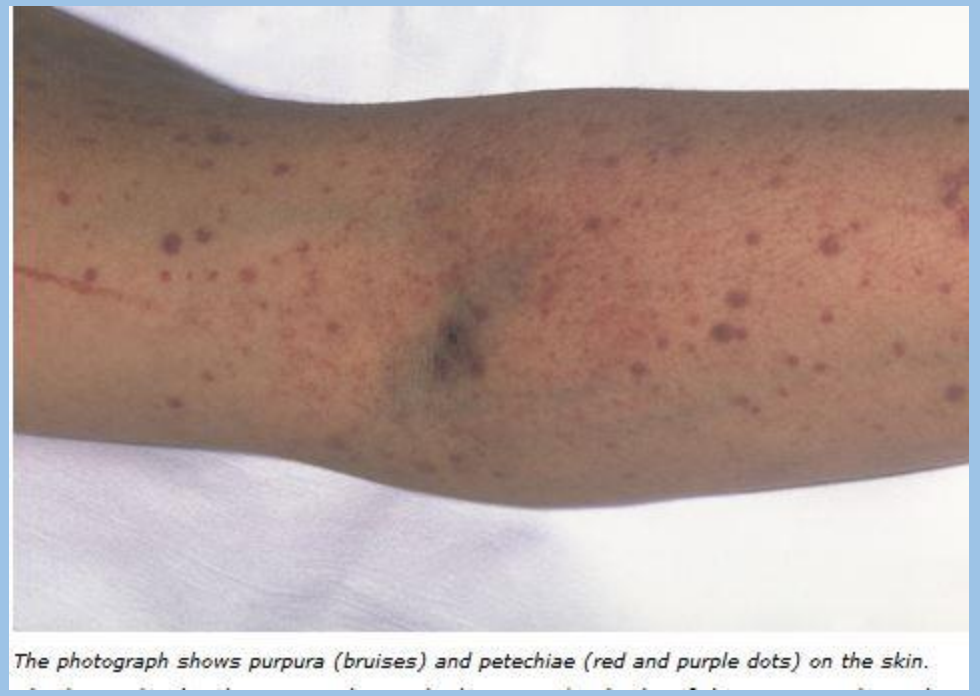
ITP - Immune thrombocytopenia

- It is an **immune-mediated platelet destruction (**involves Fc-mediated phagocytosis by macrophages via the Fcγ receptors) **along with decreased platelet production**,
- ITP is characterised by mucocutaneous bleeding episodes and **platelet count <100x10^9/L**.
- The majority of paediatric patients will spontaneously recover after therapy with higher doses of glucocorticoids,
- ITP occurs in adults less frequently, and it is often refractory to therapy with glucocorticoids.

ITP

- Signs include the spontaneous formation of petechiae , especially on the extremities, bleeding from the nostrils and/or gums, and menorrhagia (excessive menstrual bleeding), any of which may occur if the platelet count is below 20,000 per µl.
- A very low count (<10,000 per µl) may result in the spontaneous formation of hematomas (blood masses) in the mouth or on other mucous membranes. Bleeding time from minor lacerations or abrasions is usually prolonged.

ITP - spontaneous formation of petechiae



The photograph shows purpura (bruises) and petechiae (red and purple dots) on the skin.

ITP – 1st line therapy

- **High dose of corticoids (prednisone 1 mg/kg/d, dexamethasone 40 mg/d x 4, methylprednisone i.v. 1mg/kg/d)**
- **IVIG, intravenous immunoglobulin G (** 0.8 -1.0 g/kg IVIg d x 1-2) is added in case of severe bleeding.
- Rituximab. It is a chimeric monoclonal anti-CD20 antibody that depletes B lymphocytes.
- Cyclosporin A. Several small studies highlight the effectiveness of cyclosporine A for the management of refractory ITP.
- **Splenectomy -in refractory state**

Therapy of refractory ITP

- In accordance with American Society of Hematology guidelines, we **recommend observation** without treatment in most asymptomatic adults after splenectomy with a platelet count of 20 to 30 x 10^{9}/L or greater
- Only minority pts with ITP require treatment for bleeding, it usually occurring at a platelet count of < 10 x 10^{9}/L.
- **Low-dose corticosteroids:** prednisone (5 mg/d). Long-term treatment at such doses is generally well tolerated but is not devoid of risk for cumulative toxicities (diabetes mellitus, decreased bone mineral density).

Thrombocytopenia and new therapy with **thrombopoietin receptor agonists** (TRAs).

- **TRAs bind to the thrombopoietin receptor and stimulate megakaryocyte maturation and platelet production.**
- **Romiplostim** is given as a once-per-week subcutaneous injection. The starting dose is 1 mg/kg. The dose is increased by1 mg/kg each week (maximum dose 10 mg/kg) until a platelet count of 50 x 10^9/L
- **Eltrombopag** is formulated as a pill to be taken once per day. The starting dose is 50mg/d. The dose may be increased to 75mg/d to achieve a platelet count of 50 x 10^9/L

Trombocytopenia with increased destruction of platelets (microangiopathy,TTP, HIT)

Thrombotic thrombocytopenic purpura (TTP)

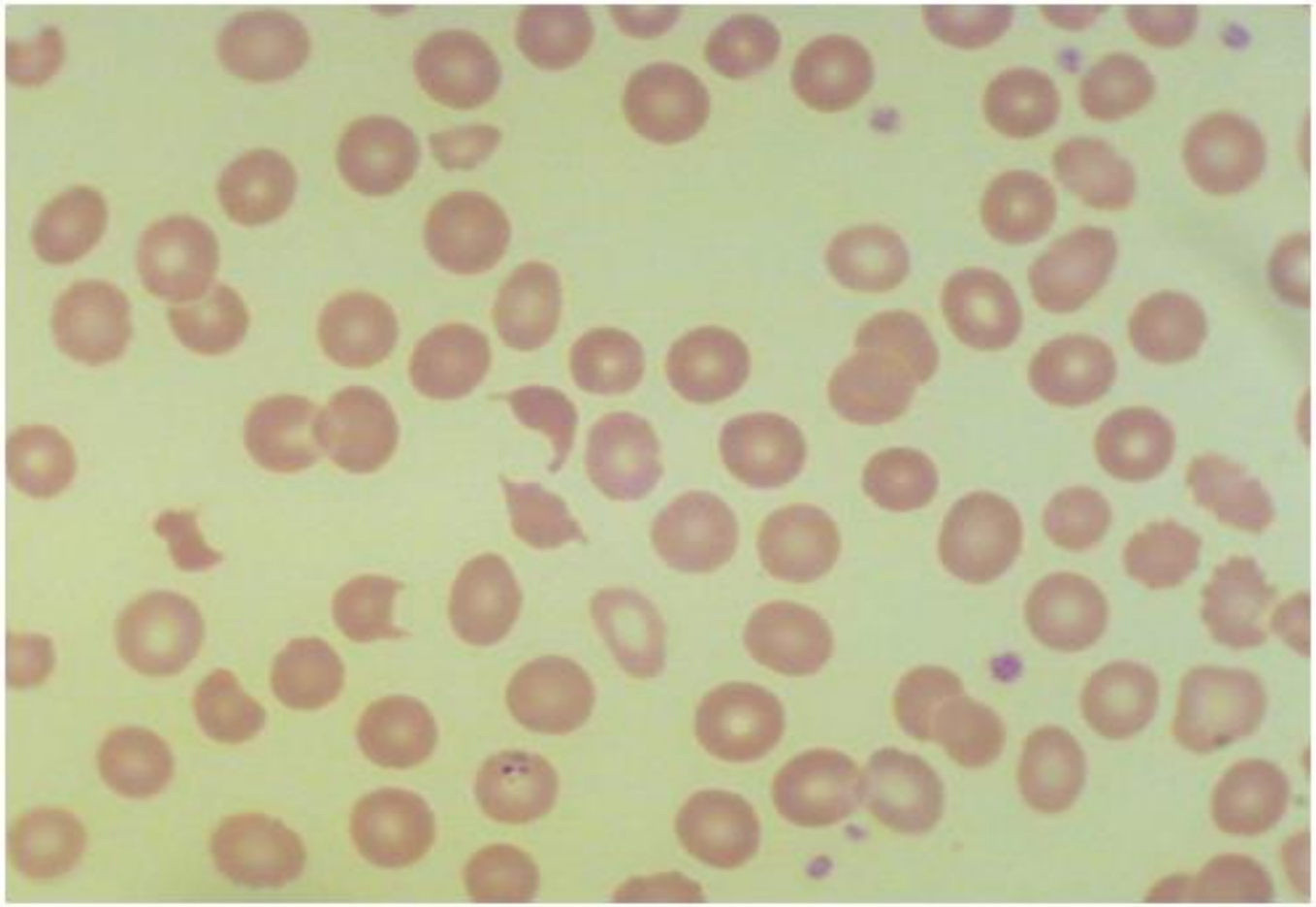
- **It is a microangiopathic disorder diagnosed by thrombocytopenia and hemolytic anemia,**

associated with a deficiency in von Willebrand factor (VWF) – cleaving **protease ADAMTS13**.

Current treatment is based on **plasma exchange**, often in combination with immunosuppressive agents.

ADAMTS 13

- it is a blood enzyme that controls the multimer size of the hemostatic protein von Willebrand factor.
- After synthesis, **ultralarge, hyperreactive** von Willebrand factor multimers (up to 20 000 kDa) are secreted from endothelial cells into the blood and are immediately **cleaved by ADAMTS13** into smaller multimers (<10 000 kDa).
- In **TTP** patients, ultralarge von Willebrand factor multimers spontaneously bind platelets, and **microthrombi are formed that block arterioles and capillaries.**
- This results in severe **organ failure**, **thrombocytopenia**, and **hemolytic anemia with schistocytes.**



Schistocytes (thrombotic thrombocytopenic purpura).

HIT – heparin induced thrombocytopenia

Antibodies aganist complex platelet factor 4 (cationt) and heparin (aniont)

4T Scoring System for Evaluating the Pretest Probability of Heparin-Induced Thrombocytopenia.

Table 1. 4T Scoring System for Evaluating the Pretest Probability of Heparin-Induced Thrombocytopenia.*

Variable	Score		
	2	1	0
Acute thrombocytopenia	Platelet count decrease of >50% and nadir ≥20,000/mm^3	Platelet count decrease of 30–50% or nadir 10,000–19,000/mm^3	Platelet count decrease of <30% or nadir ≤10,000/mm^3
Timing of onset	Day 5–10, or day 1 if recent heparin exposure	>Day 10 or unclear exposure	≤Day 4 with no recent heparin exposure
Thrombosis	New thrombosis or anaphylactoid reaction after heparin bolus	Progressive or recurrent thrombosis	None
Other cause of thrombocytopenia	None	Possible	Definite
Total score	6–8, indicating high score	4 or 5, indicating intermediate score	0–3, indicating low score

* Adapted from Lo et al.[31] A low 4T score (0 to 3 points) has a high negative predictive value. The day that heparin was started is considered as day 0. The onset of heparin-induced thrombocytopenia (HIT) is defined as the day that the platelet count begins to decrease. Patients in whom the score is difficult to apply, owing to missing platelet count values or coexisting conditions causing thrombocytopenia, and those with an intermediate or high score require further evaluation. This score can be included on ordering forms for HIT laboratory testing (e.g., www2.medizin.uni-greifswald.de/transfus/fileadmin/user_upload/doku_thrombo_gerinnung/platelet_lab_request_form.pdf).

Greinacher A. N Engl J Med 2015;373:252-261.

Heparin-Induced Thrombocytopenia Treatment & Management

- If heparin-induced thrombocytopenia (HIT) is suspected, the first step is to discontinue all heparin products immediately and avoid any further exposure.
- Patients with HIT are at high risk for thrombotic events and should be treated with alternative anticoagulants, typically a direct thrombin inhibitor (DTI). The US Food and Drug Administration (FDA) has approved the DTI **argatroban**.
- The indirect factor Xa inhibitor fondaparinux (Arixtra) is approved for use in HIT, especially in stable, non–critically ill patients.

DIC syndrome and thrombocytopenia

- Disseminated intravascular coagulation (DIC) is a condition characterized by systemic activation of coagulation, potentially leading to thrombotic obstruction of small and midsize vessels, thereby contributing to organ dysfunction.
- At the same time, ongoing **consumption of platelets** and coagulation proteins **results in thrombocytopenia** and low concentrations of clotting factors, which may cause profuse hemorrhagic complications.

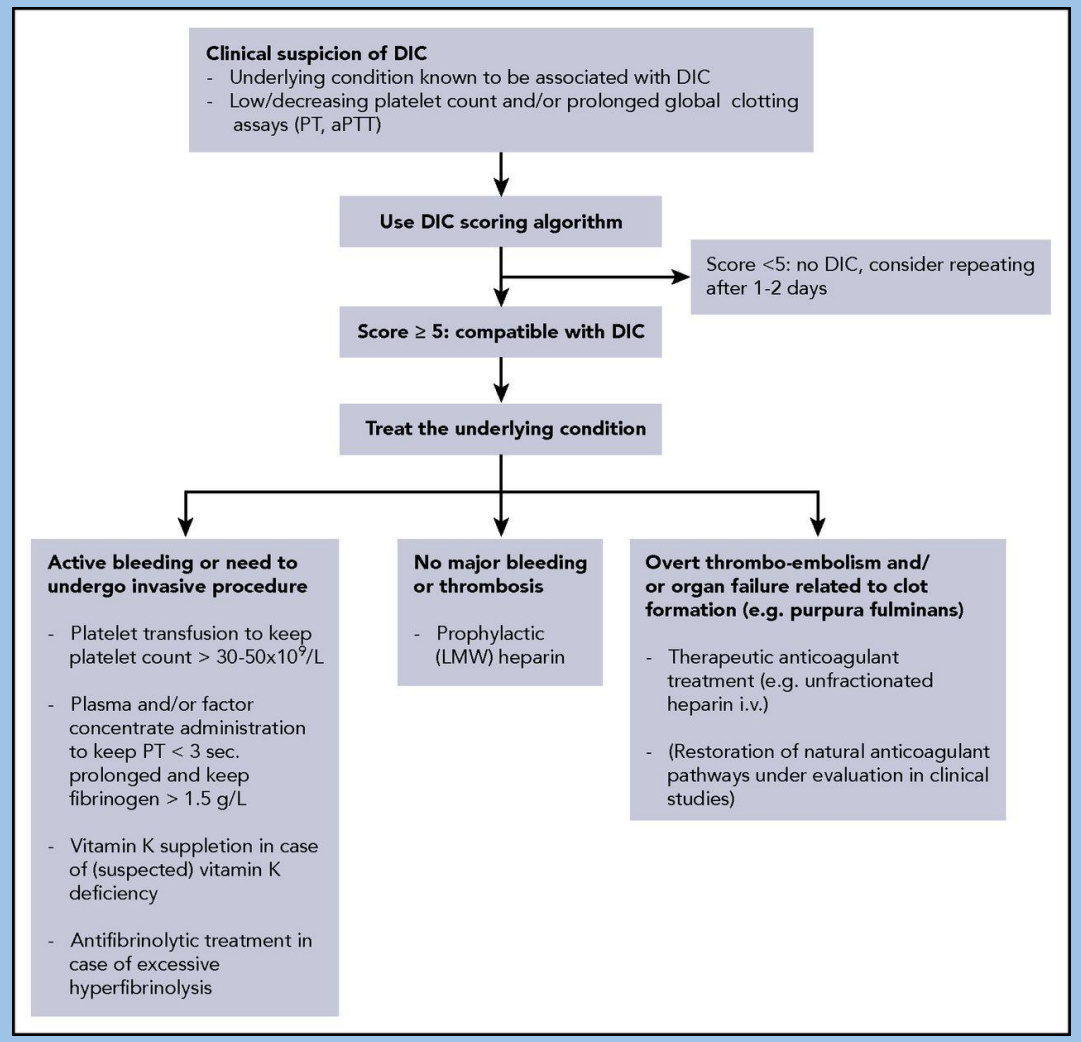
Scoring algorithm for the diagnosis of DIC

Platelet count, ×10⁹/L >100 = 0 <100 = 1 <50 = 2
Level of fibrin markers (eg D-dimer, fibrin degradation products) No increase = 0 Increased but <5x upper limit of normal = 2 Strong increase (≥5x upper limit of normal) = 3
Prolonged prothrombin time* <3 s = 0 ≥3 s but <6 s = 1 ≥ 6 s = 2
Fibrinogen level >1.0 g/L = 0 ≤1.0 g/L = 1

This scoring system is only appropriate in patients with an underlying disorder that can be associated with DIC. A score of ≥5 points is compatible with DIC. Note that if the score is <5, consider repeating after 1 to 2 days.[43]

*If prothrombin time values are only available as INRs, an INR value of >1.3 or >1.5 will generate 1 or 2 points, respectively (assuming the International Sensitivity Index value of the thromboplastin reagents used is close to 1).

Flowchart for the diagnostic and therapeutic management of DIC.

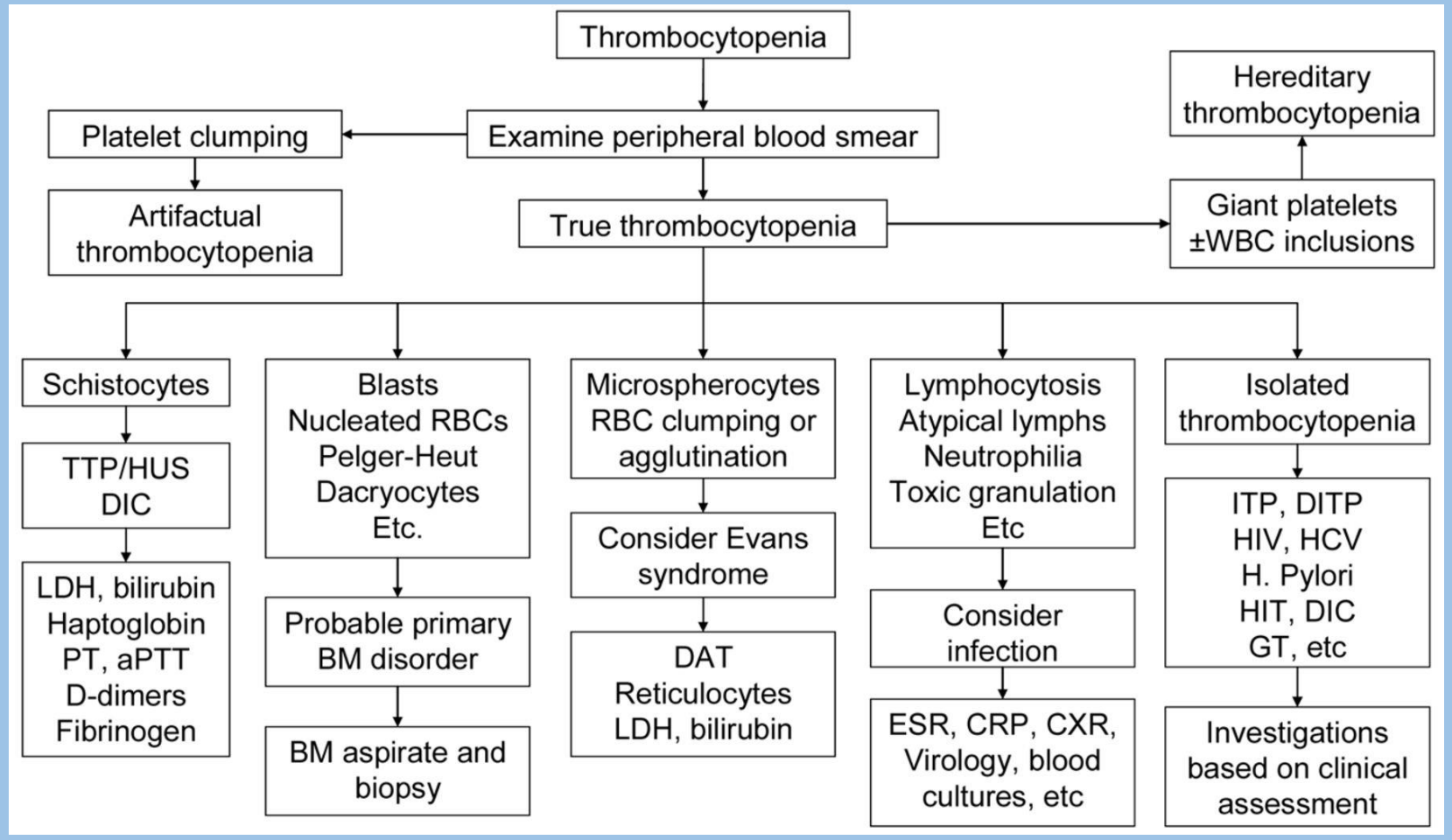


Marcel Levi, and Marie Scully Blood 2018;131:845-854

DIC sy with consumption of plateles (in meningocal sepsis)

- **HELLP syndrome** is a life-threatening pregnancy complication usually considered to be a variant or complication of pre-eclampsia.
- HELLP" is an abbreviation of the three main features of the syndrome:**H**emolysis, **E**levated **L**iver enzymes, and **L**ow **P**latelet count.
- The syndrome may be associated with serious liver manifestations, including infarction, **consumptions of platelets** with hemorrhage, and rupture of placenta.

Algorithm for workup of thrombocytopenia based on the observation of the peripheral blood film.

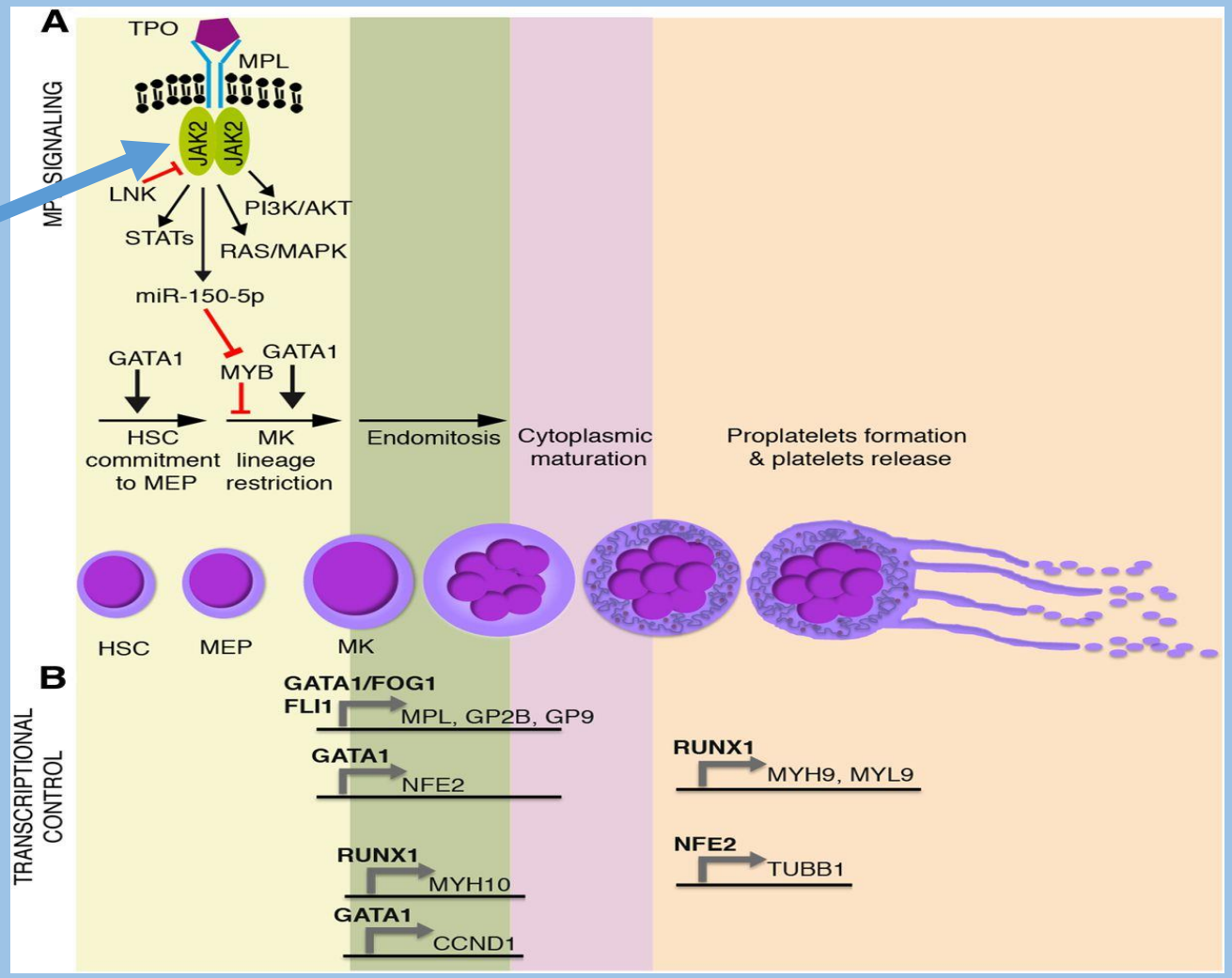


Terry Gernsheimer et al. Blood 2013;121:38-47

B.
Thrombocytosis and thrombocythemia
platelet count > 450 x 10^9/l

- **1) Reactive**
  - Chronic infection
  - Chronic inflammation
  - Malignancy
  - Hyposplenism **(post-splenectomy)**
  - Iron deficiency
  - Acute blood loss
- **2) Myeloproliferative neoplasms (Ph-) :** platelets are both elevated and activated
  - Essential thrombocytosis
  - Polycythemia vera

Main molecular mechanisms affected in malignant megakaryopoiesis and platelet function defects – JAK - 2 V617F gene mutation.

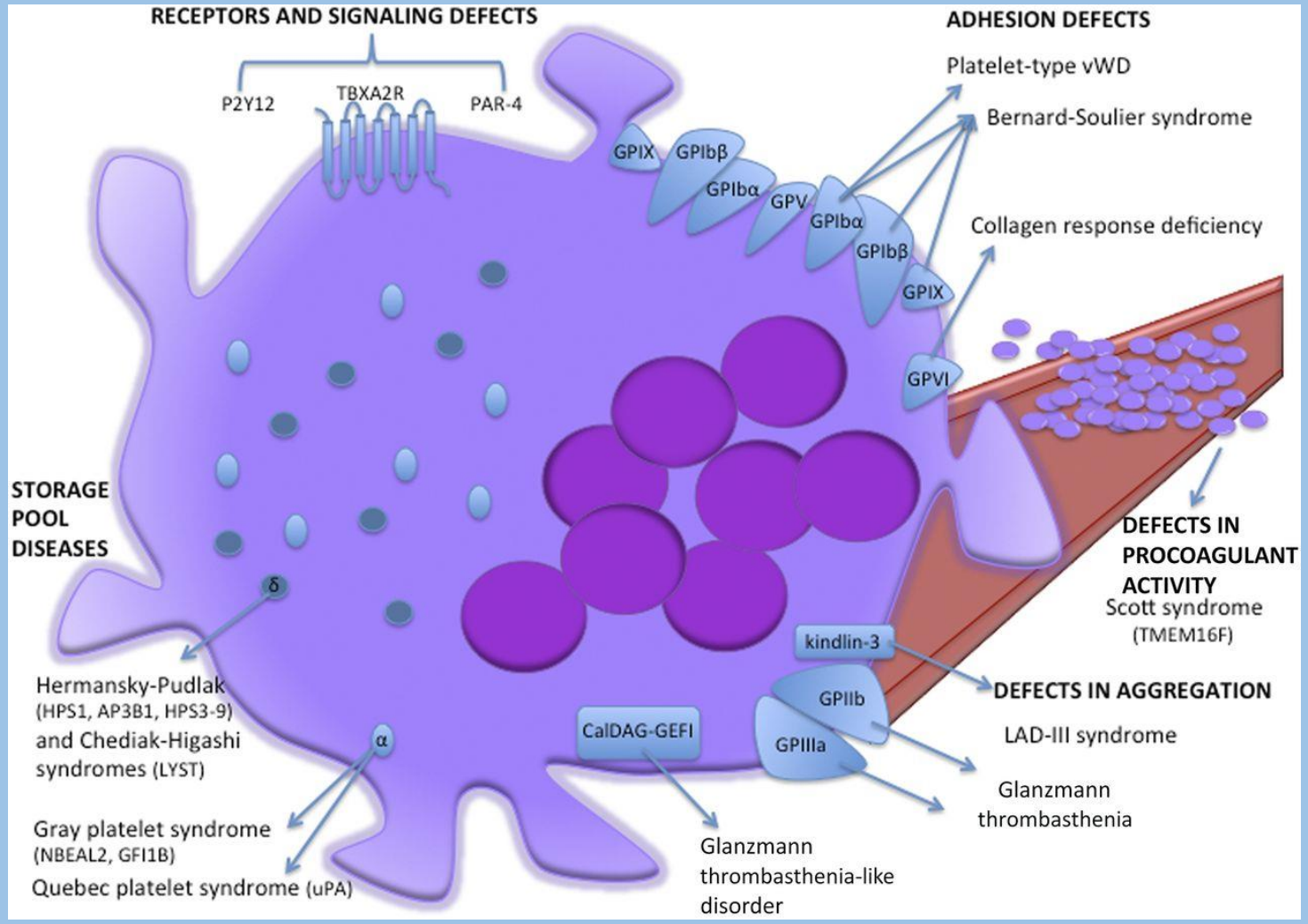


Elisa Bianchi et al. Blood 2016;127:1249-1259

Altered platelet function-thrombocytopathy

- Congenital
  - Disorders of adhesion
    - Bernard-Soulier syndrome
  - Disorders of activation
    - Disorders of granule release
    - Hermansky-Pudlak Syndrome
  - Disorders of aggregation
    - Glanzmann's thrombasthenia

Schematic cartoon representing the proteins mutated in inherited platelet function disorders.



Elisa Bianchi et al. Blood 2016;127:1249-1259

Hermansky-Pudlak syndrome - defects in granule content / storage pool deficiencies

- Hermansky-Pudlak syndrome is inherited as an autosomal recessive disorder with associated **occulocutaneous albinism**.
- It is characterized as a mild bleeding disorder with prolonged bleeding time and a **marked absence of dense bodies**.
- Platelet function studies show an absent secondary wave to ADP, epinephrine, and ristocetin, and abnormal aggregation with collagen.

Prof. MUDr. František Heřmanský, DrSc.

(* 22. 2. 1916 Praha, † 8. 12. 1980 Praha)

head of 1st Dept of internal medicine, 1st Faculty of medicine, Charles University at Prague

- **Albinism Associated with Hemorrhagic Diathesis and Unusual Pigmented Reticular Cells in the Bone Marrow: Report of Two Cases with Histochemical Studies**
- F. HERMANSKY and P. PUDLAK
- Blood 1959 14:162-169

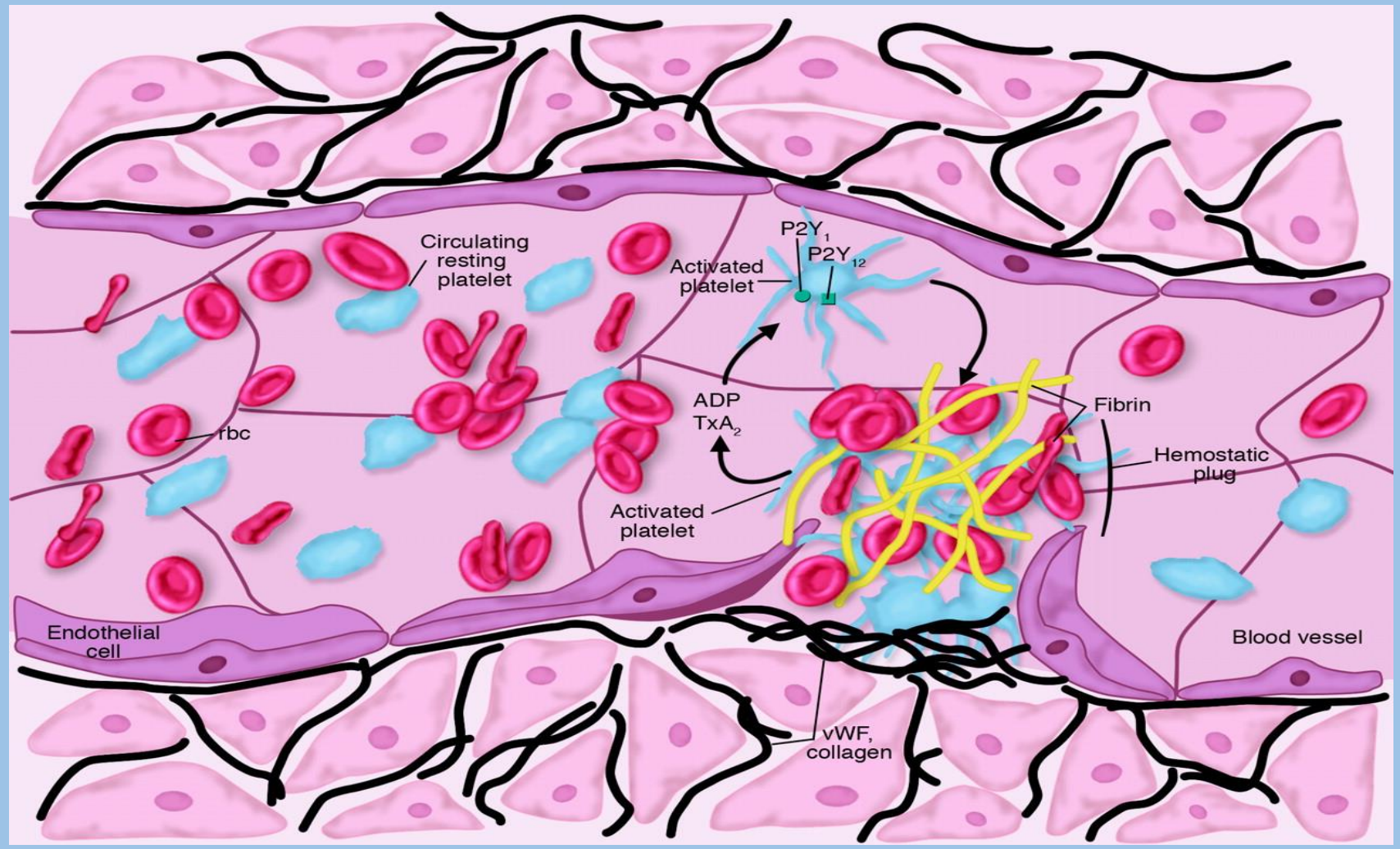
Secondary thrombocytopathy –

- after treatment with antiplatelet drugs

These drugs are among the **most used drugs in cardiology.**

They are indicated for the prevention of arterial (platelet) thrombus formation.

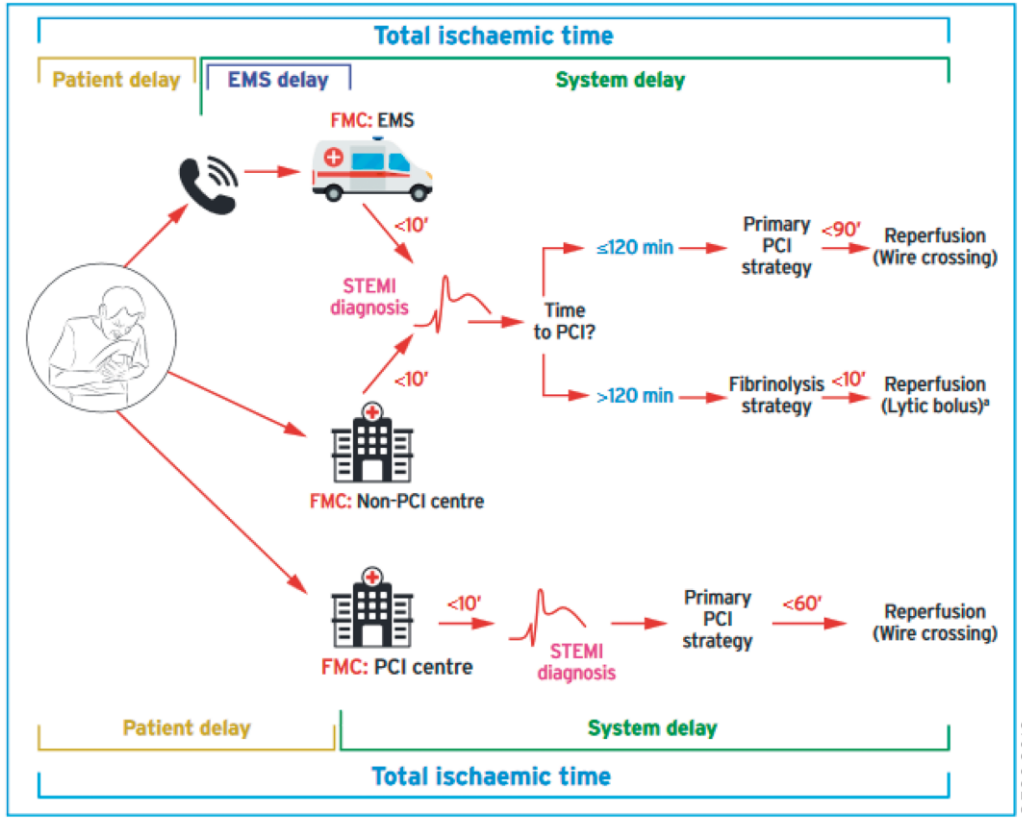
Platelets in arterial thrombogenesis



Patients who require the use of **antiplatelet drugs** are:

- **Stroke** with or without atrial fibrillation,
- **Heart surgery** (after by-pass surgery , prosthetic replacement heart valve),
- **Coronary Heart Disease** : stable angina, unstable angina and heart attack, patients with coronary stent,
- **Peripheral Vascular Disease**/Peripheral Arterial Disease

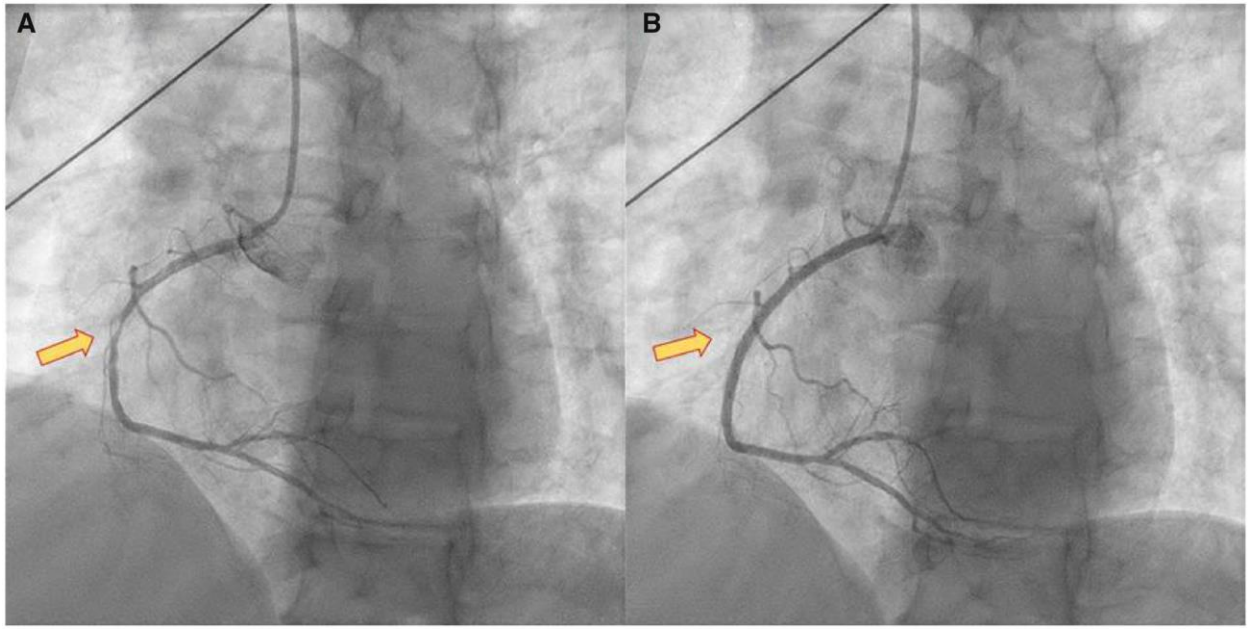
Figure 5 Modes of patient's medical contact, components of ischaemia time, and flowchart for reperfusion strategy ...



The recommended mode of patient presentation is by alerting the EMS (call national emergency number: 112 or similar number according to region). When STEMI diagnosis is made in the out-of-hospital setting (via EMS) or in a non-PCI centre, the choice of reperfusion strategy is based on the estimated time from STEMI diagnosis to PCI-mediated reperfusion (wire crossing). System delay for patients alerting the EMS starts at the time of phone alert, although FMC occurs when the EMS arrives at the scene. EMS = emergency medical service.
FMC = first medical contact; PCI = percutaneous coronary intervention; STEMI = ST-segment elevation myocardial infarction. 'denotes minutes.
[a]Patients receiving fibrinolysis should be transferred to a PCI centre immediately after administration of the lytic bolus.

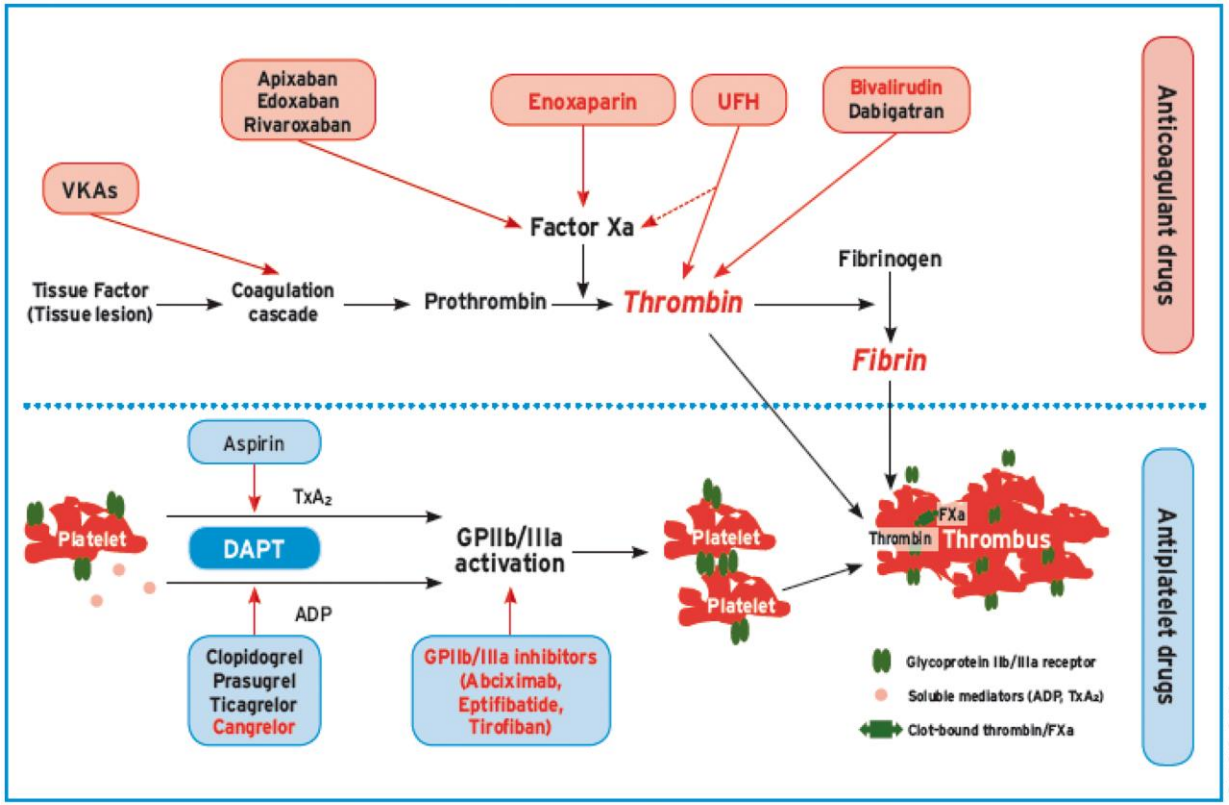
European Heart Journal, Volume 40, Issue 2, 07 January 2019, Pages 87–165, https://doi.org/10.1093/eurheartj/ehy394

Figure 2 Coronary angiography showing significant stenosis of mid-right coronary artery (A) and a successful stenting ...



European Heart Journal Supplements, Volume 21, Issue Supplement_G, November 2019, Pages G7–G9,
https://doi.org/10.1093/eurheartj/suz192

Figure 9 Antithrombotic treatment for myocardial revascularization and its pharmacological targets.



The figure illustrates anticoagulant and antiplatelet drugs being used during and after myocardial revascularization (percutaneous coronary intervention or coronary artery bypass grafting). Drugs with oral administration are shown in black letters and drugs with preferred parenteral administration in red.
ADP = adenosine diphosphate; DAPT = dual antiplatelet therapy; FXa = factor Xa; GP = glycoprotein; TxA_2 = thromboxane A_2; UFH = unfractionated heparin; VKAs = vitamin K antagonists.

European Heart Journal, Volume 40, Issue 2, 07 January 2019, Pages 87–165, https://doi.org/10.1093/eurheartj/ehy394

Main classes of antiplatelets drugs

Cyclooxygenase Inhibitors

- Aspirin

Antagonists of ADP receptor

- Ticlopidin
- Clopidogrel
- Prasugrel (Efient)
- Ticagrelor

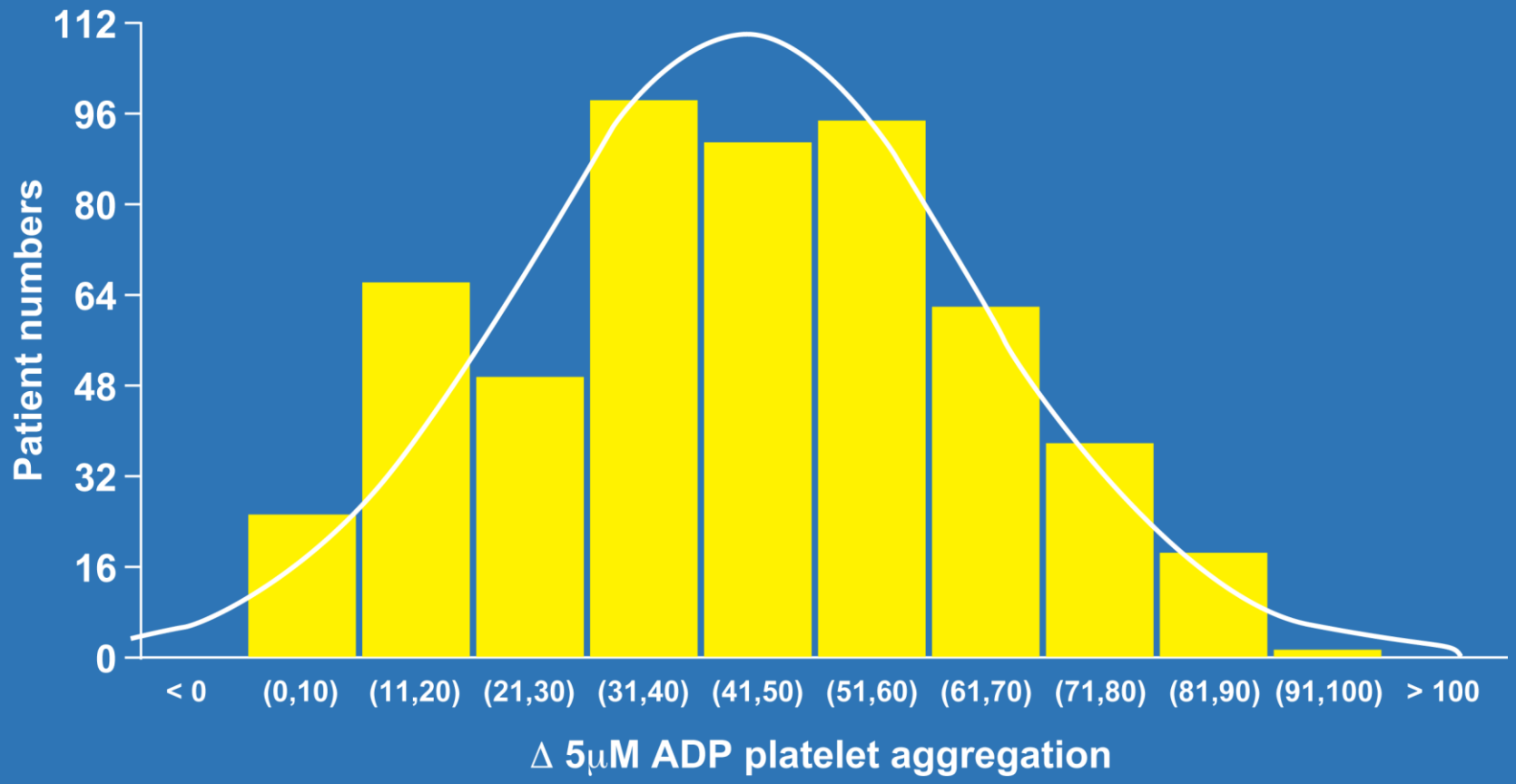
Antagonists of GP IIb/IIIa

- Abciximab (Reopro)
- Tirofiban (Aggrastat)
- Eptifibatide (Integrilin)

ADP receptor antagonists

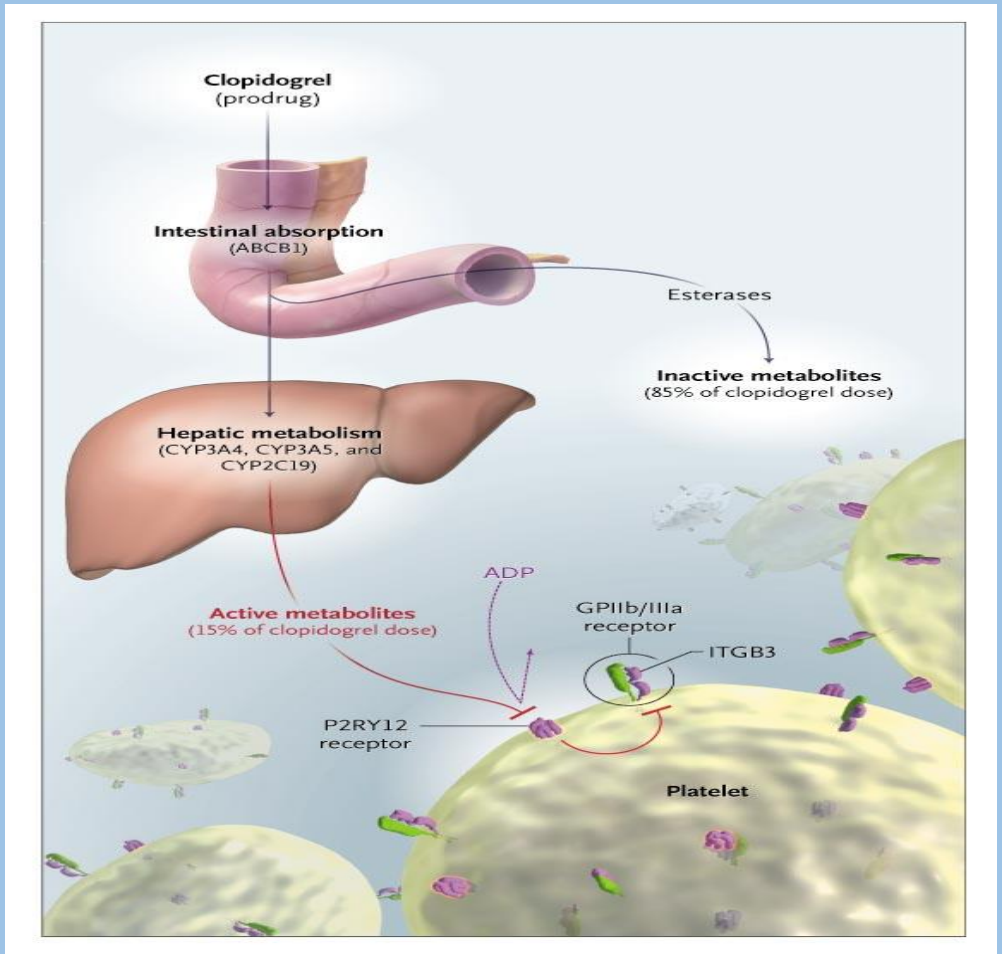
Drug	Administration		Prodrug	ttPeak plt inhibition	Reversibility (half-life)
	Route	Frequency			
Clopidogrel	Oral	Once daily	yes	2-6h (after 600 mg loading dose)	No
Prasugrel	Oral	Once daily	yes	2h	No
Ticagrelor	Oral	Twice daily	No	2h	**Yes (12h)**

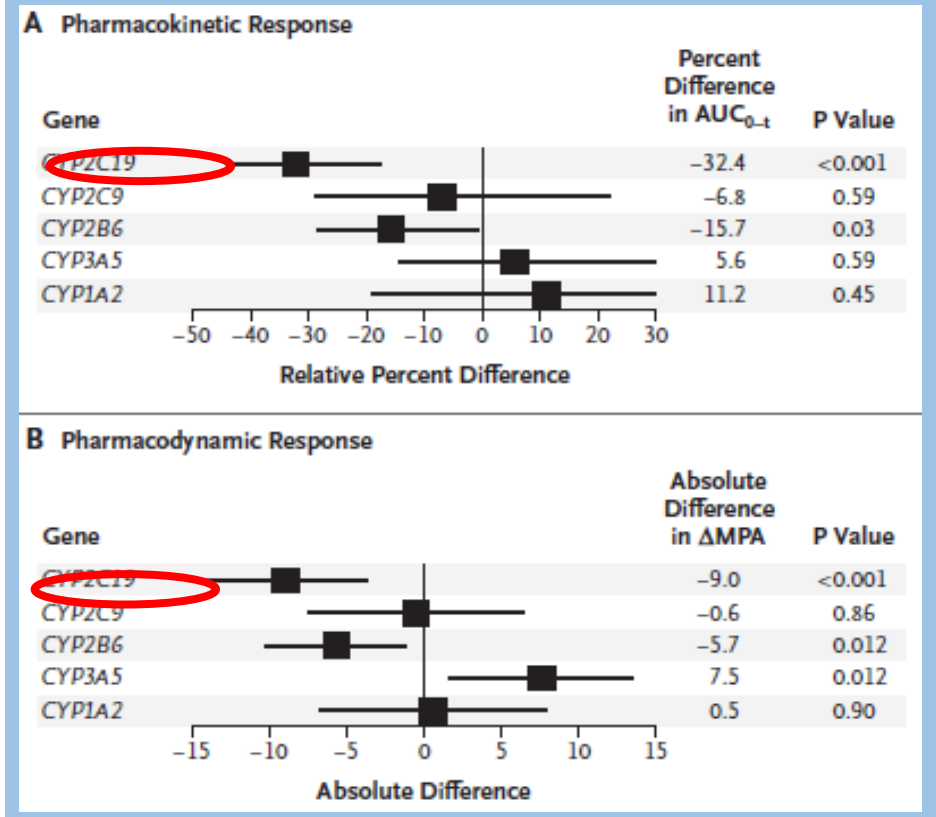
Problem - Variability of clopidrogel response



Serebruany VL, Steinhubl SR, Berger PB, et al. JACC 2005; 45:246 –51.

Metabolism of clopidogrel – hereditary infuence of CYP 2C 19 mutations





Simon T. et al. N EJM 2008; Megat et al. NEJM 2008.

Cytochrom P450 CYP2C19

chí-kvadrát (p 0,7254)

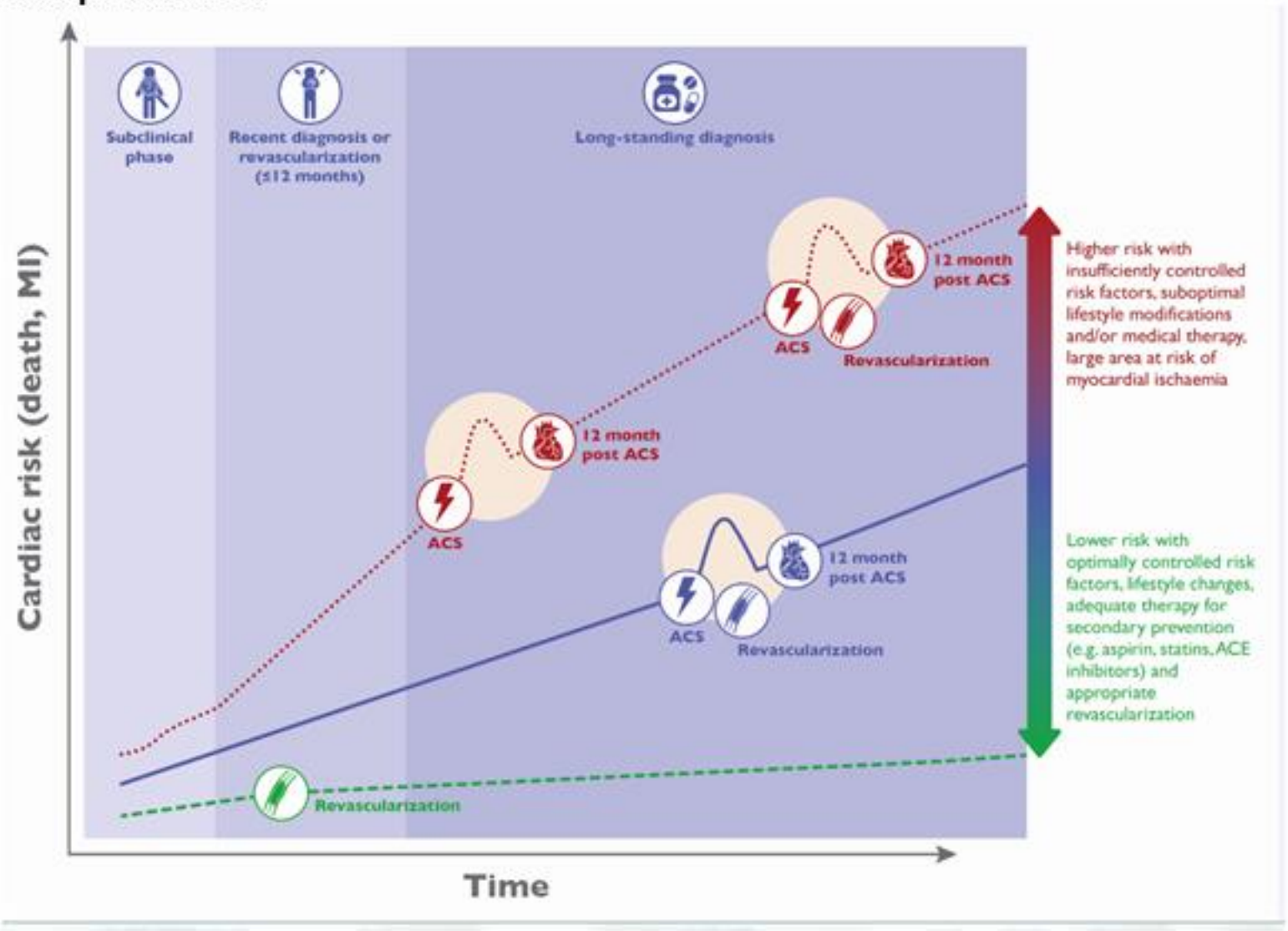
genotyp	prevalence v ČR dárci krve (n 1450)	u nemocných se stabilní AP (n 696)
***1/*1**	**74,48%**	**72,41%**
***1/*2**	**22,83%**	**25.14%**
***1/*3**	**0,14%**	**0,14%**
***2/*2**	**2,28%**	**1,87%**
***3/*3**	**0,00%**	**0,00%**
***2/*3**	**0,00%**	**0,00%**

Recommendations for platelet inhibition in non-ST-elevation acute coronary syndromes

Recommendations	Class[a]	Level[b]	Ref.[c]
Oral antiplatelet therapy			
Aspirin is recommended for all patients without contraindications at an initial oral loading dose[d] of 150–300 mg (in aspirin-naive patients) and a maintenance dose of 75–100 mg/day long-term regardless of treatment strategy.	I	A	129–132
A $P2Y_{12}$ inhibitor is recommended, in addition to aspirin, for 12 months unless there are contraindications such as excessive risk of bleeds.	I	A	137, 148, 153
• Ticagrelor (180 mg loading dose, 90 mg twice daily) is recommended, in the absence of contraindications,[e] for all patients at moderate-to-high risk of ischaemic events (e.g. elevated cardiac troponins), regardless of initial treatment strategy and including those pretreated with clopidogrel (which should be discontinued when ticagrelor is started).	I	B	153
• Prasugrel (60 mg loading dose, 10 mg daily dose) is recommended in patients who are proceeding to PCI if no contraindication.[e]	I	B	148, 164
• Clopidogrel (300–600 mg loading dose, 75 mg daily dose) is recommended for patients who cannot receive ticagrelor or prasugrel or who require oral anticoagulation.	I	B	137

Natural history of chronic coronary syndromes

A dynamic process



ESC Guidelines on the diagnosis and management of chronic coronary syndromes (European Heart Journal 2019; 10.1093/eurheartj/ehz425)

Patients with angina and/or dyspnoea and coronary artery disease - Event prevention (1)

Recommendations	Class	Level
Antithrombotic therapy in patients with CCS and in sinus rhythm		
Aspirin 75-100 mg daily is recommended in patients with a previous MI or revascularization.	I	A
Clopidogrel 75 mg daily is recommended as an alternative to aspirin in patients with aspirin intolerance.	I	B
Clopidogrel 75 mg daily may be considered in preference to aspirin in symptomatic or asymptomatic patients, with either PAD or a history of ischaemic stroke or transient ischaemic attack.	IIb	B
Aspirin 75-100 mg daily may be considered in patients without a history of MI or revascularization, but with definitive evidence of CAD on imaging.	IIb	C

Patients with angina and/or dyspnoea and coronary artery disease - Event prevention (2)

Recommendations	Class	Level
Antithrombotic therapy in patients with CCS and in sinus rhythm		
Adding a second antithrombotic drug to aspirin for long-term secondary prevention should be considered in patients with high risk of ischaemic events[a] and without high bleeding risk.[b]	IIa	A
Adding a second antithrombotic drug to aspirin for long-term secondary prevention may be considered in patients with at least a moderately increased risk of ischaemic events[c] and without high bleeding risk.[b]	IIb	A

[a] Diffuse multivessel CAD with at least one of the following: diabetes mellitus requiring medication, recurrent MI, PAD, or CKD with eGFR 15-59 mL/min/1.73 m^2.
[b] Prior history of intracerebral haemorrhage or ischaemic stroke, history of other intracranial pathology, recent gastrointestinal bleeding or anaemia due to possible gastrointestinal blood loss, other gastrointestinal pathology associated with increased bleeding risk, liver failure, bleeding diathesis or coagulopathy, extreme old age or frailty, or renal failure requiring dialysis or with eGFR <15 mL/min/1.73 m^2.
[c] At least one of the following: multivessel/diffuse CAD, diabetes mellitus requiring medication, recurrent MI, PAD, HF, or CKD with eGFR 15-59 mL/min/1.73 m2

Chronic coronary syndromes and atrial fibrillation

- Oral anticoagulants (OAC) + antiplatelet drugs

What is new in the 2019 Guidelines?

New recommendations (2)

ESC European Society of Cardiology

Antithrombotic therapy in patients with CCS and sinus rhythm	Antithrombotic therapy in patients with CCS and atrial fibrillation	Antithrombotic therapy in post-PCI patients with indication for OAC
Adding a second antithrombotic drug to aspirin for long-term secondary prevention in patients with high-risk of ischaemic events and without high bleeding risk.	NOAC is recommended in preference to a VKA.	Full dose NOAC is recommended in preference to a VKA.
	Long-term OAC therapy in patients with AF and a CHA_2DS_2-VASc score ≥2 in males and ≥3 in females.	Rivaroxaban 15 mg over 20 mg.
		Dabigatran 110 mg over 150 mg.
		Early cessation (≤1 week) of aspirin.
Adding a second antithrombotic drug to aspirin for long-term secondary prevention in patients with at least a moderately increased risk of ischaemic events and without high bleeding risk.	Long-term OAC therapy in patients with AF and a CHA_2DS_2-VASc score 1 in males and 2 in females.	Triple therapy for 1 to 6 months.
		INR 2.0-2.5 and TTR >70% if VKA.
		Dual therapy with prasugrel or ticagrelor over clopidogrel.

Class I Class IIa Class IIb Class III

ESC Guidelines on the diagnosis and management of chronic coronary syndromes (European Heart Journal 2019; 10.1093/eurheartj/ehz425)

Patients with angina and/or dyspnoea and coronary artery disease - Event prevention (5)

Recommendations	Class	Level
Antithrombotic therapy in patients with CCS and AF		
When oral anticoagulation is initiated in a patient with AF who is eligible for a NOAC,[a] a NOAC is recommended in preference to a VKA.	I	A
Long-term OAC therapy (NOAC or VKA with time in therapeutic range >70%) is recommended in patients with AF and a CHA_2DS_2-VASc score[b] ≥2 in males and ≥3 in females.	I	A

[a] See Summary of Product Characteristics for reduced doses or contraindications for each NOAC in patients with CKD, body weight <60 kg, age >75–80 years, and/or drug interactions.
[b] Congestive HF, hypertension, age ≥75 years (2 points), diabetes, prior stroke/transient ischaemic attack/embolus (2 points), vascular disease (CAD on imaging or angiography, prior MI, PAD, or aortic plaque), age 65-74 years, and female sex.

problems of antithrombotic therapy : thrombosis : bleeding