
بسم الله الرحمن الرحيم

Thrombosis in Hemoglobinopathies: Mechanisms, Challenges, and Therapeutic Perspectives

Dr. Bijan Keikhaei
Thalassemia and Hemoglobinopathy Research Center, Health Research Institute, Ahvaz Jundishapur

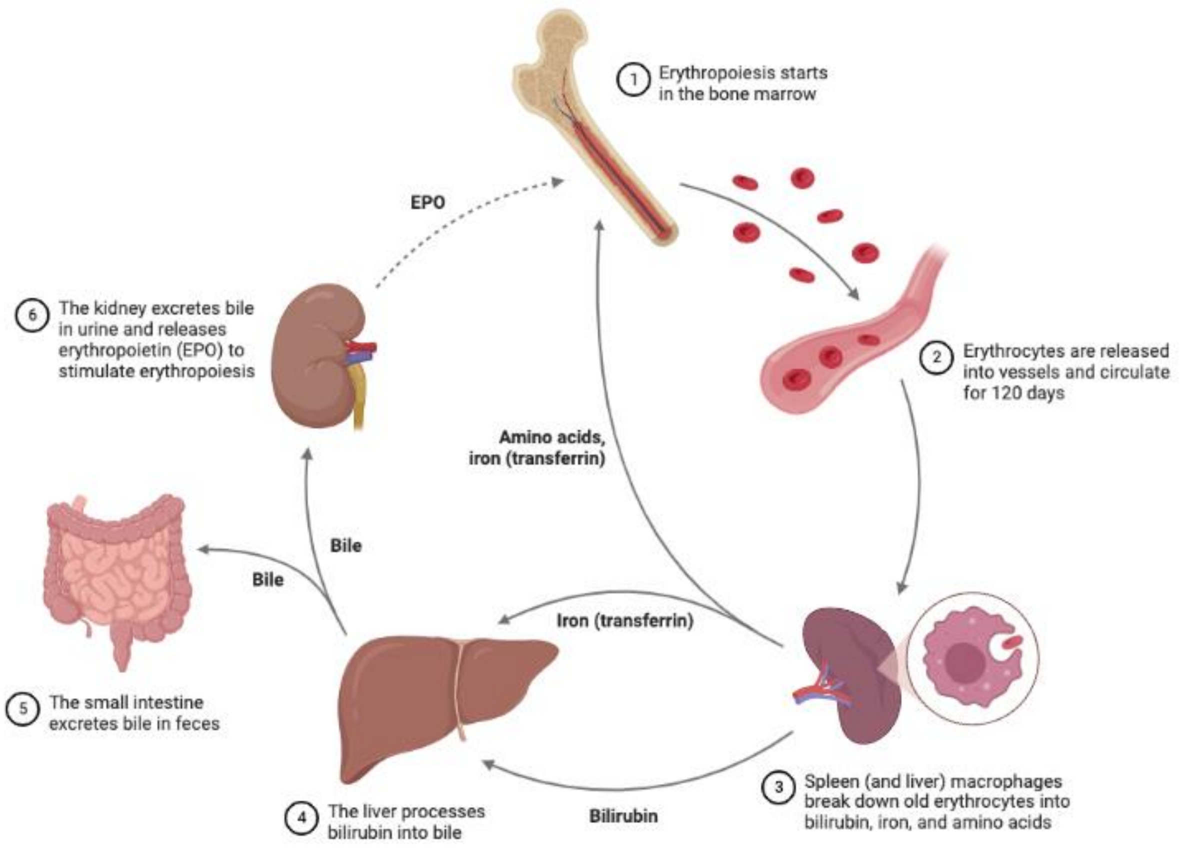
Normal RBC Physiology

- Approximately 2.5 million RBCs are produced per second under normal conditions.
- Under stress or hypoxia, erythropoiesis can increase up to 8-fold.
- Each RBC contains ~450 million hemoglobin molecules, each carrying four heme groups, and each heme binds one oxygen molecule.
- Iron (Fe^{2+}) serves as the core atom in heme because it can bind oxygen reversibly, allowing oxygen loading in the lungs and release in tissues. Each gram of hemoglobin binds about 1.34 mL of O_2, so 100 mL of blood (with ~15 g Hb) can carry approximately 20 mL of oxygen.

Normal RBC Physiology

- RBC lifespan (~120 days)
- Travels >500 km during its life
- Role in oxygen transport and gas exchange
- Spleen → mechanical filter, old RBC removal
- Heart and kidney medulla → shear stress and microcirculation

RBC Passages by Organ



Organ	Circulation Type	Estimated Passages During RBC Lifespan
Aorta	Single systemic passage (left ventricular outflow)	≈ **172,800** passages
Kidney	Parallel circulation (~20%)	≈ **34,500** passages
Spleen	Parallel circulation (~3–5%)	≈ **5,000–8,600** passages

RBC Sick Syndrome

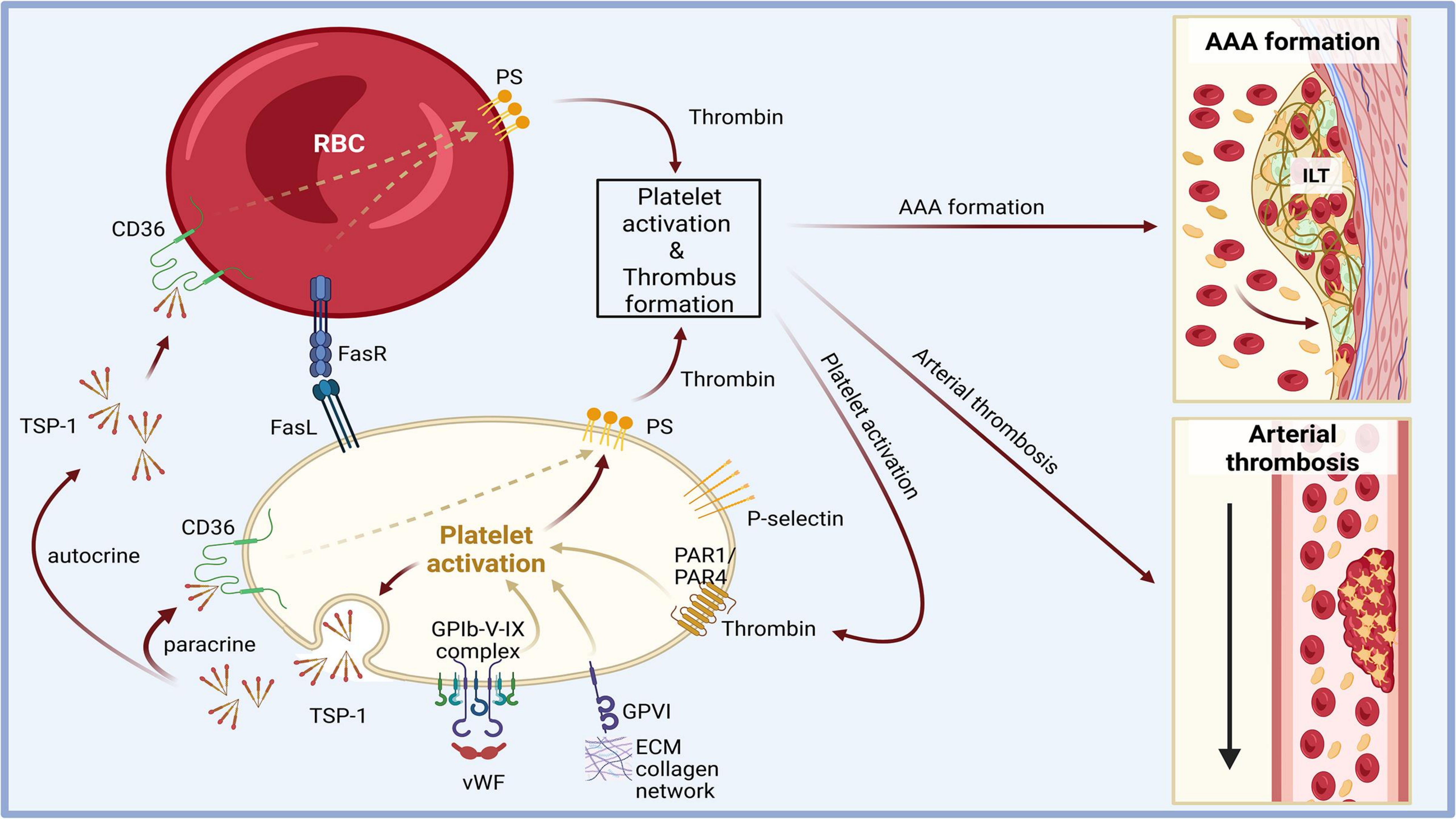
- **Definition**: functional and structural abnormalities due to chronic hemolysis (e.g., SCD, thalassemia)
- Altered deformability, membrane instability , sticky (adhesive), pro-inflammatory

Phosphatidylserine Exposure and Microparticles

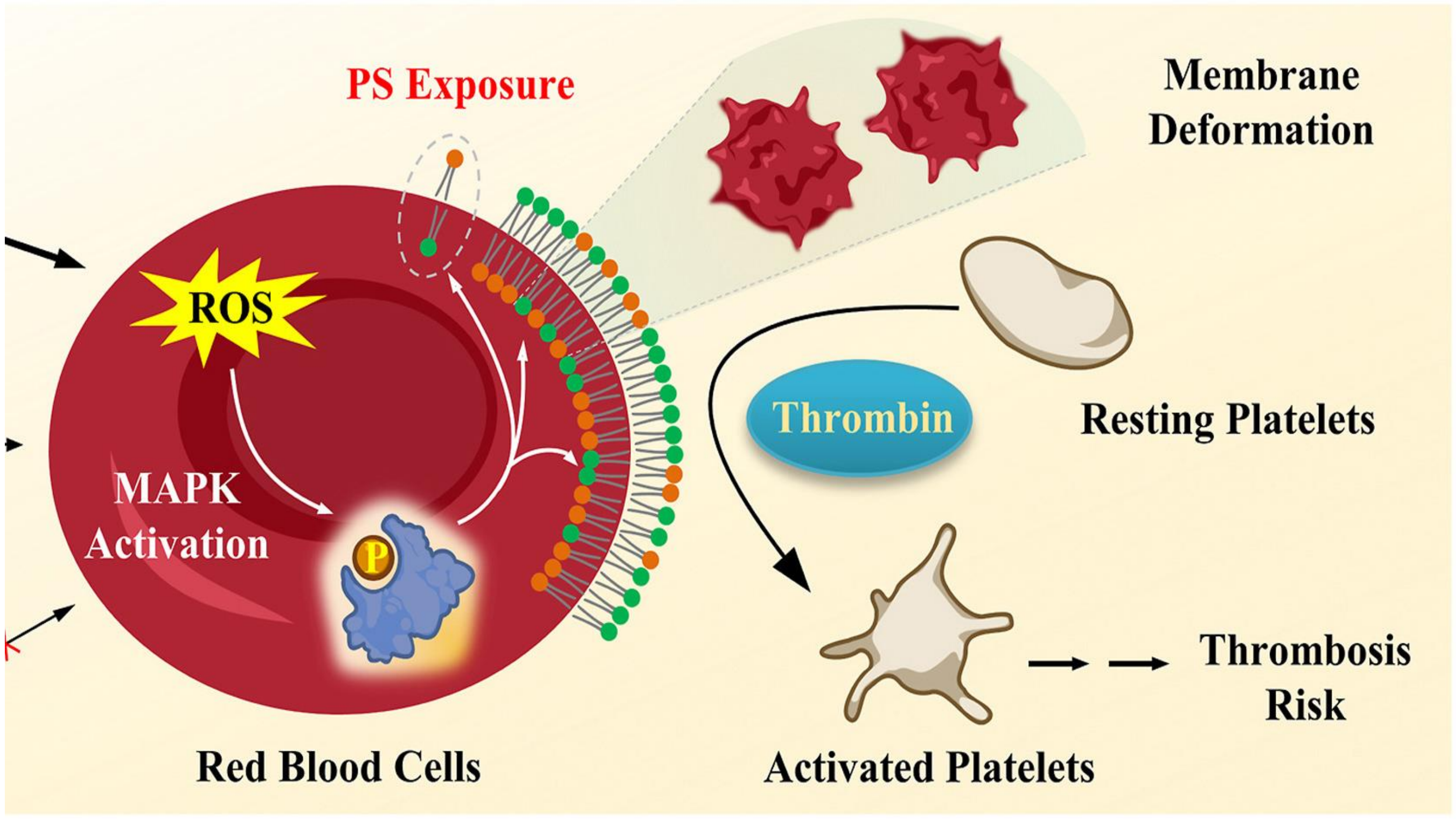
- Normally, Phosphatidylserine (PS) is confined to the inner leaflet of the RBC membrane. Upon oxidative or mechanical stress, PS flips outward. Exposed PS binds factors **Va, Xa** → prothrombinase complex formation.

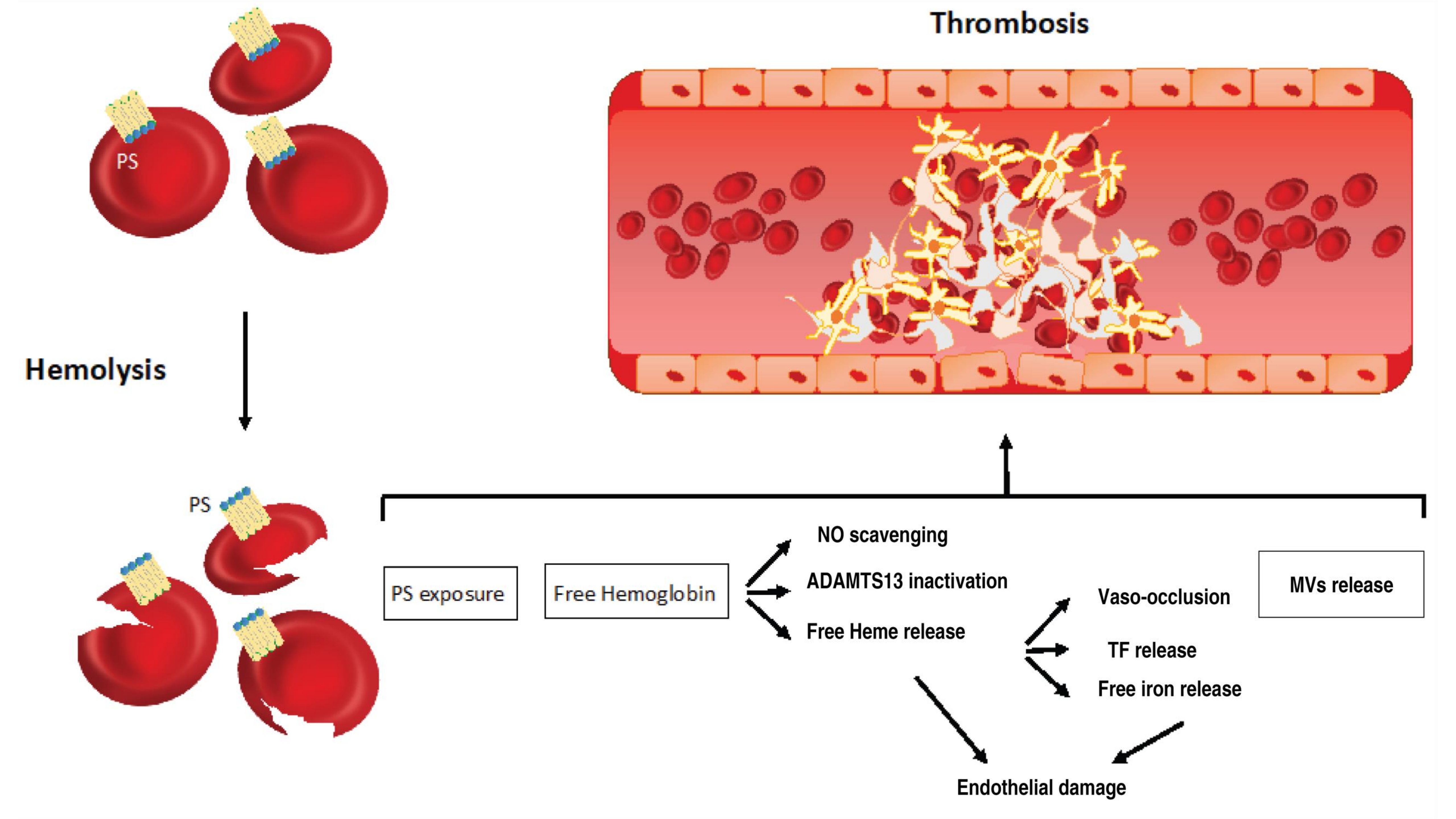
- RBC-derived microparticles (RMPs) circulate post-hemolysis and amplify coagulation. Seen in SCD, β-thalassemia, and post-splenectomy states.

- Microparticle released from RBC, Endothelial cell, PLT, Leukocyte (Monocyte)

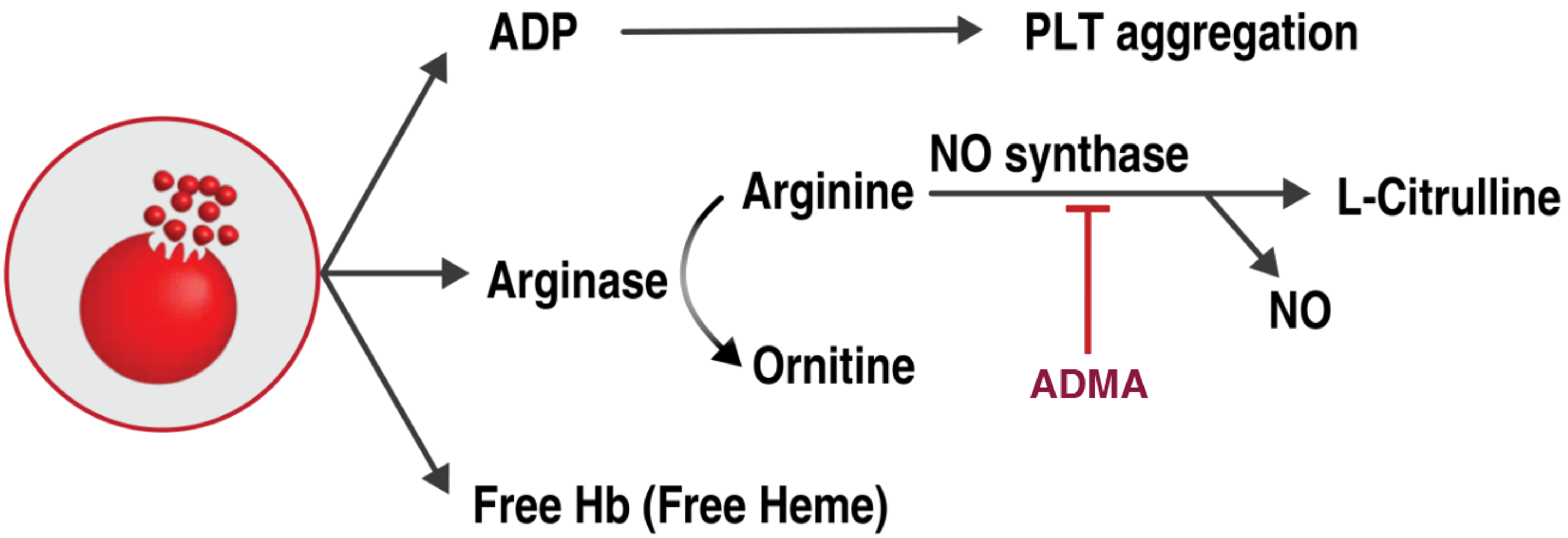


RBC
PS
Thrombin
CD36
FasR
FasL
TSP-1
Platelet activation & Thrombus formation
AAA formation
AAA formation
ILT
Thrombin
PS
Platelet activation
Arterial thrombosis
Arterial thrombosis
P-selectin
CD36
autocrine
Platelet activation
PAR1/ PAR4
Thrombin
paracrine
GPIb-V-IX complex
TSP-1
GPVI
ECM collagen network
vWF

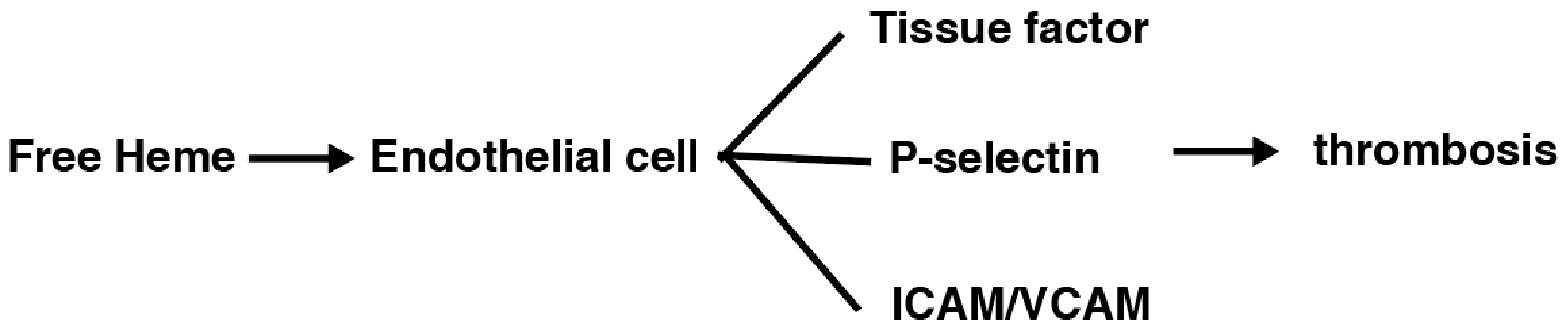

PS Exposure
Membrane
Deformation
ROS
MAPK
Activation
P
Thrombin
Resting Platelets
Thrombosis
Risk
Red Blood Cells
Activated Platelets


Thrombosis
PS
Hemolysis
PS
PS exposure
Free Hemoglobin
NO scavenging
ADAMTS13 inactivation
Free Heme release
Vaso-occlusion
TF release
Free iron release
MVs release
Endothelial damage

Endothelial dysfunction syndrome



Endothelial dysfunction syndrome



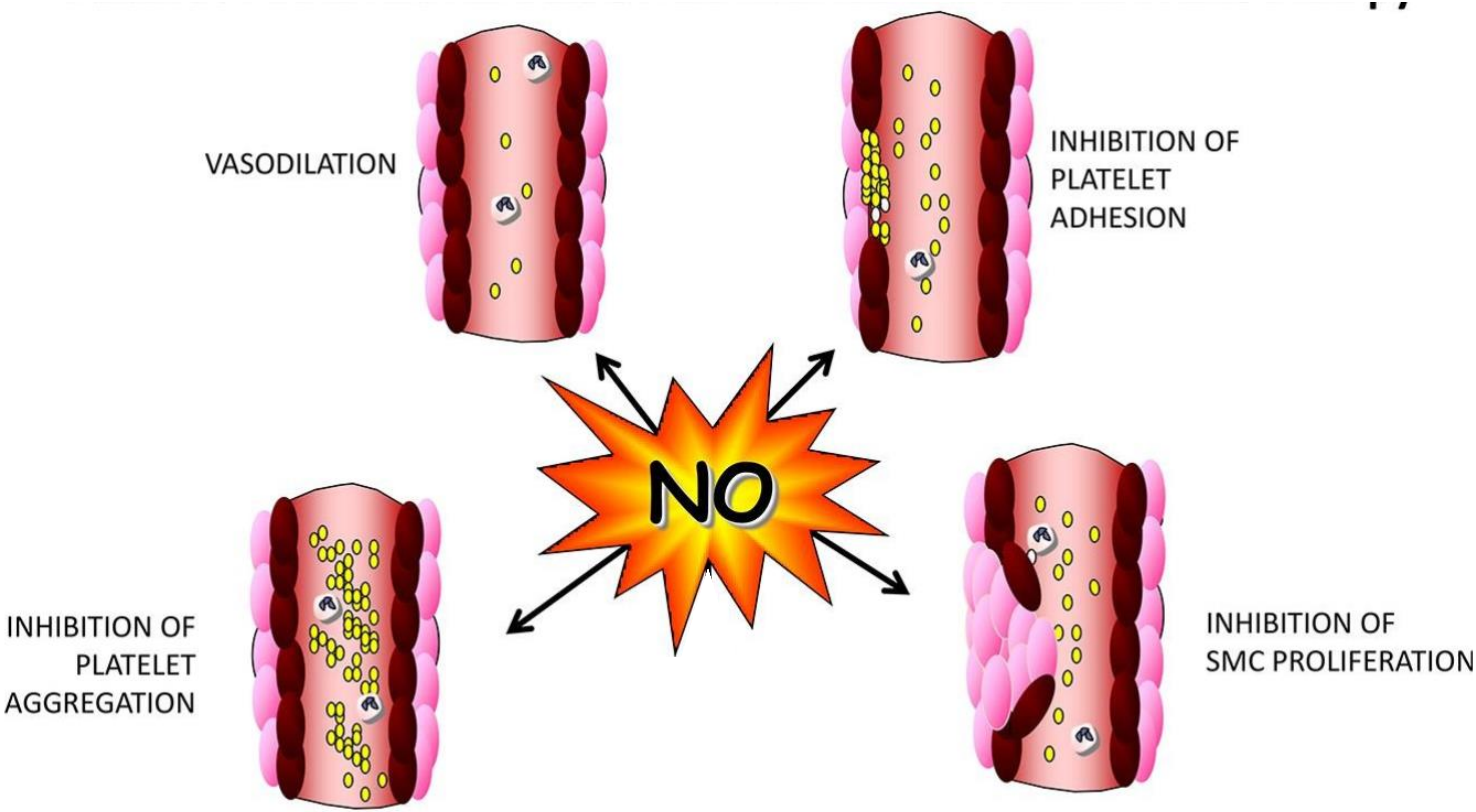
- ADAMT13 Depletion

The Role of Nitric Oxide (NO)

- NO depletion in hemolysis results from:
  Free hemoglobin (Hb) acting as a potent NO scavenger
  Reduced L-arginine availability (NO substrate)

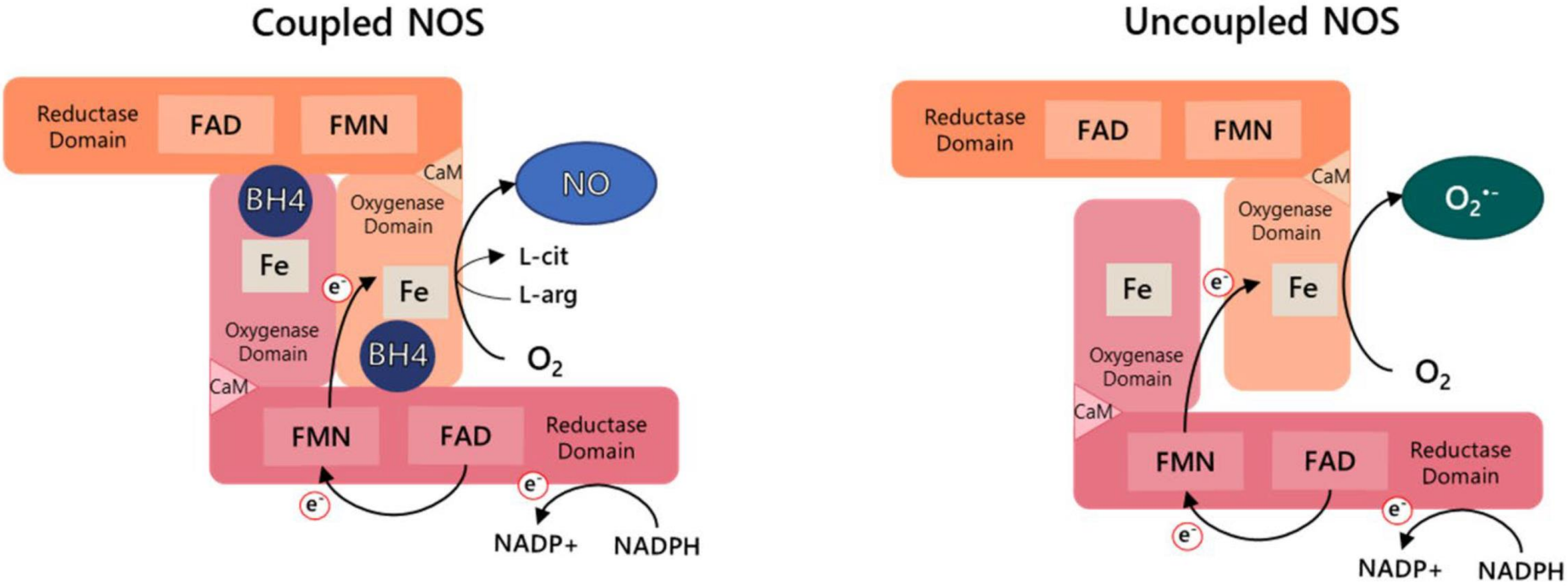
- Consequences of NO depletion:
  Enhanced platelet aggregation
  Increased tissue factor expression
  Vasoconstriction and endothelial activation

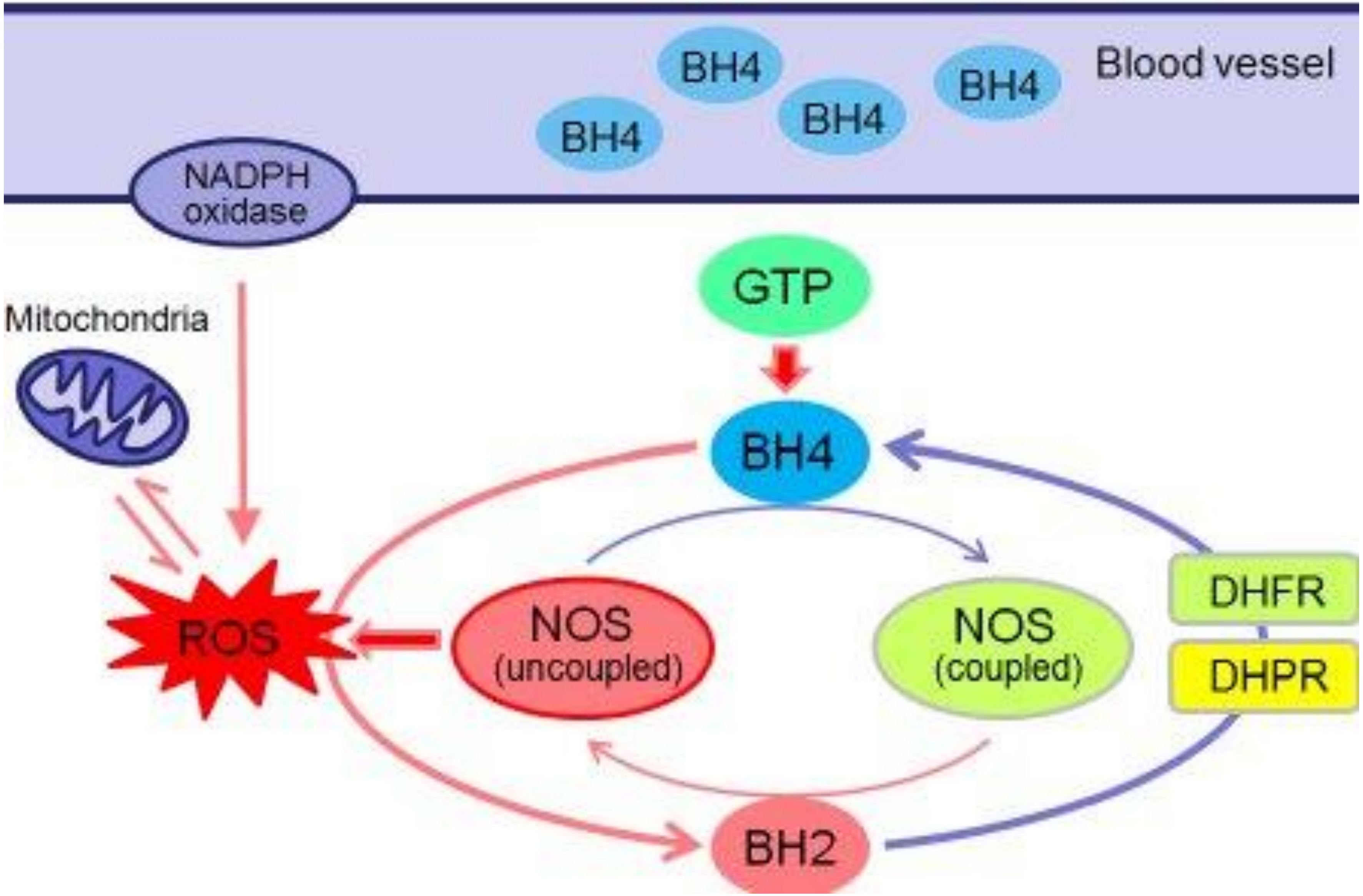
- Additional note:
  Nitric oxide (NO) facilitates the binding of ADAMTS13, which consequently leads to ADAMTS13 dysfunction.


VASODILATION
INHIBITION OF PLATELET ADHESION
NO
INHIBITION OF PLATELET AGGREGATION
INHIBITION OF SMC PROLIFERATION

NOS (Nitric Oxide Synthase)

- **NOS isoforms:** eNOS, nNOS, and iNOS convert L-arginine → NO + L-citrulline
- **Cofactor**: Tetrahydrobiopterin (BH4) (also known as biopterin or Vitamin H_4) is essential for proper NOS coupling



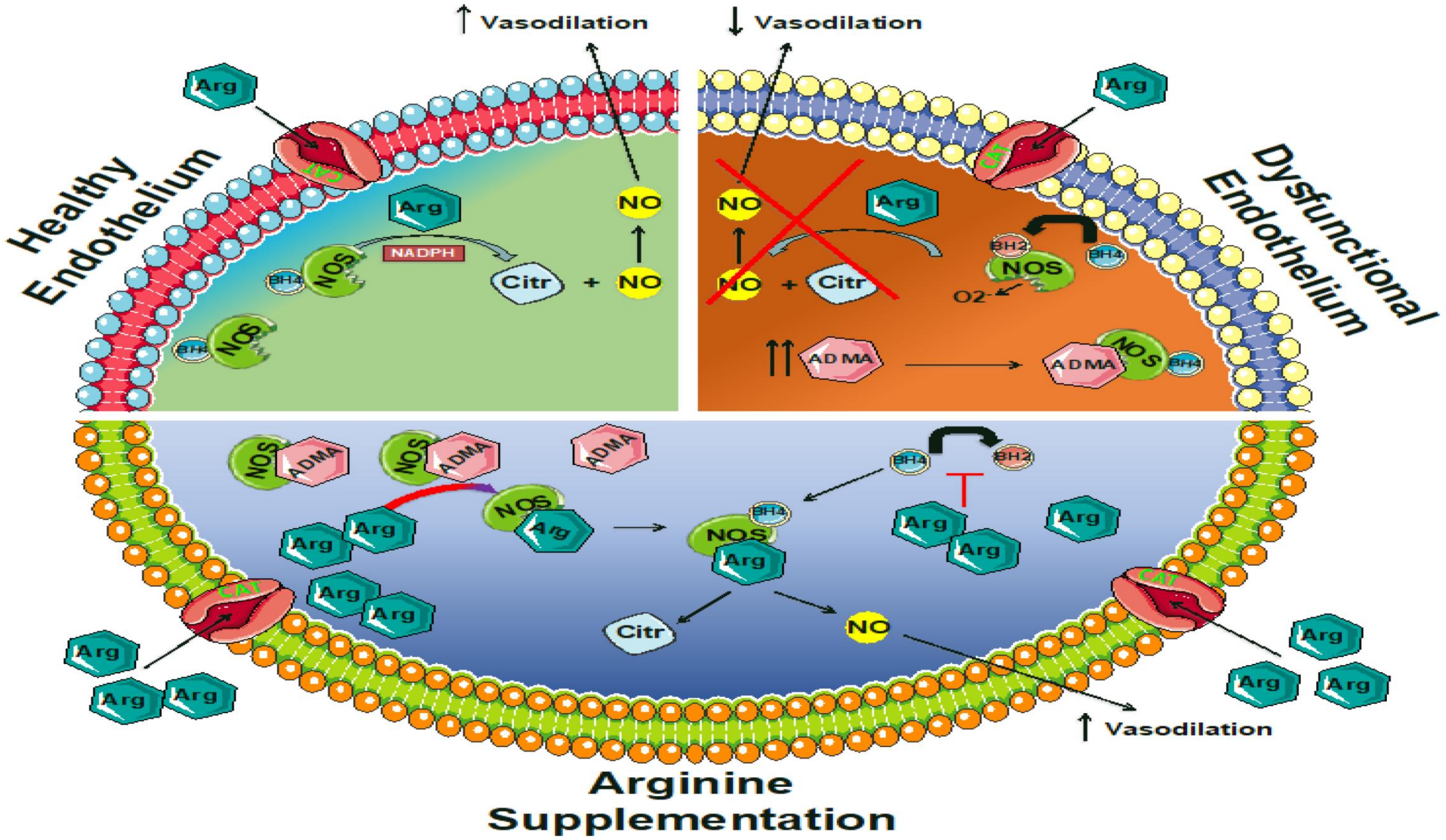

BH4
BH4
BH4
BH4
Blood vessel
NADPH oxidase
Mitochondria
GTP
BH4
ROS
NOS (uncoupled)
NOS (coupled)
DHFR
DHPR
BH2

BH4 (Vitamin H4)

- **BH4 deficiency:**
  Causes NOS uncoupling → Superoxide (O_2^-) generation → Oxidative stress
  Leads to endothelial dysfunction and reduced NO bioavailability

  - **In hemoglobinopathies:**
    Chronic hemolysis and ROS oxidize BH4 → **BH2**
    Resulting in impaired NO signaling and prothrombotic endothelial activation

❖ Therapeutic note: BH4 or L-arginine supplementation can restore eNOS function and attenuate thrombosis risk.



↑ Vasodilation
↓ Vasodilation
Healthy Endothelium
Dysfunctional Endothelium
Arg
CAT
NOS
BH4
NADPH
Citr
NO
BH2
O2-
ADMA
↑ Vasodilation
Arginine Supplementation

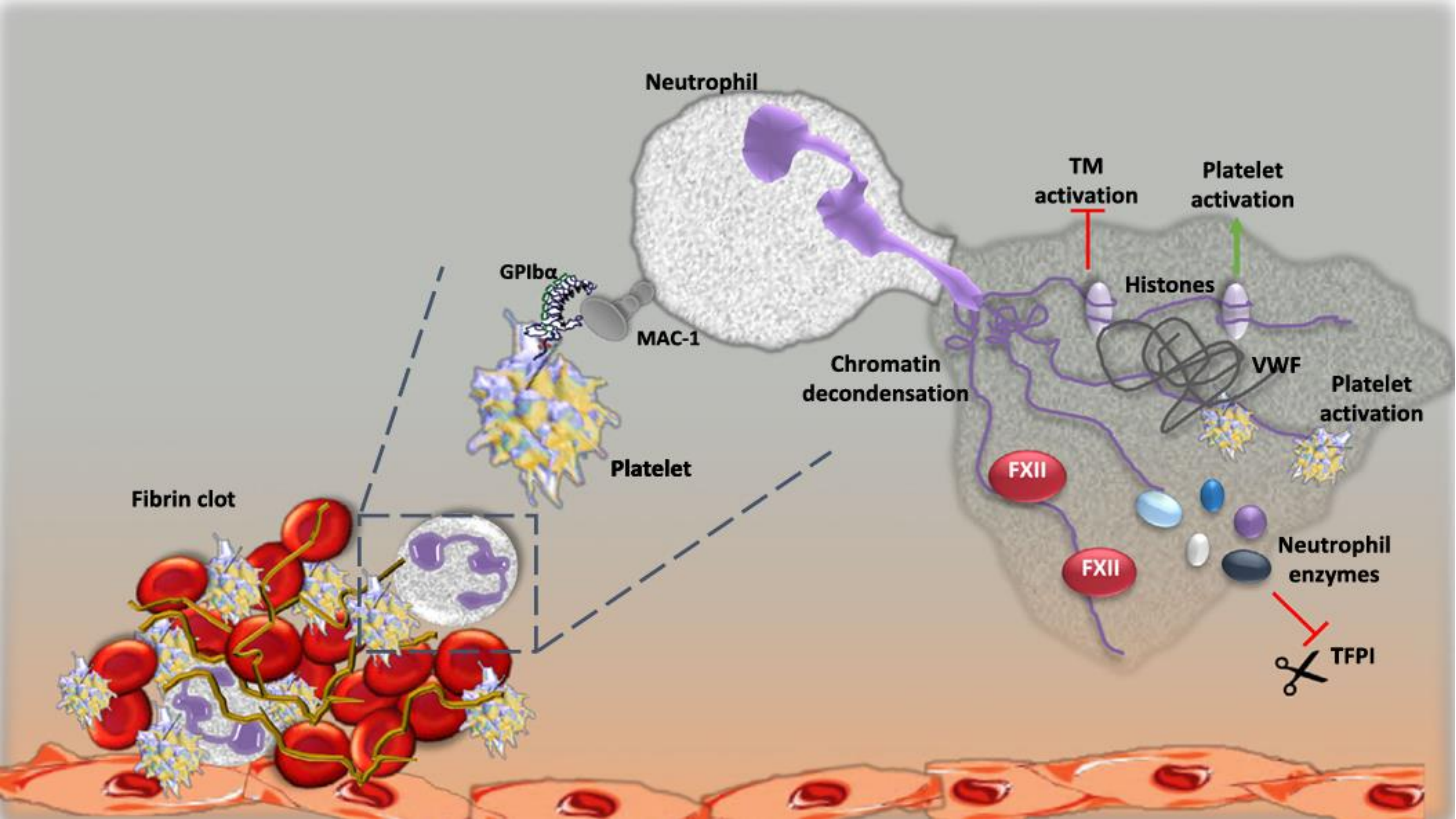
The HINT Pathway: Hemolysis → Inflammation → NETosis → Thrombosis

H= Hemolysis: releases free Hb and heme → oxidative injury and inflammation

I= Inflammation: heme >> DAMP >> NF-kb >>> inflammation , ROS

N= NETosis: ROS >>> neutrophils release extracellular traps (NETs), containing proteases

T= Thrombosis: NETs + PS + microparticles create a prothrombotic scaffold



Neutrophil
TM
activation
Platelet
activation
GPIbα
MAC-1
Histones
Chromatin
decondensation
VWF
Platelet
activation
Platelet
FXII
Fibrin clot
FXII
Neutrophil
enzymes
TFPI

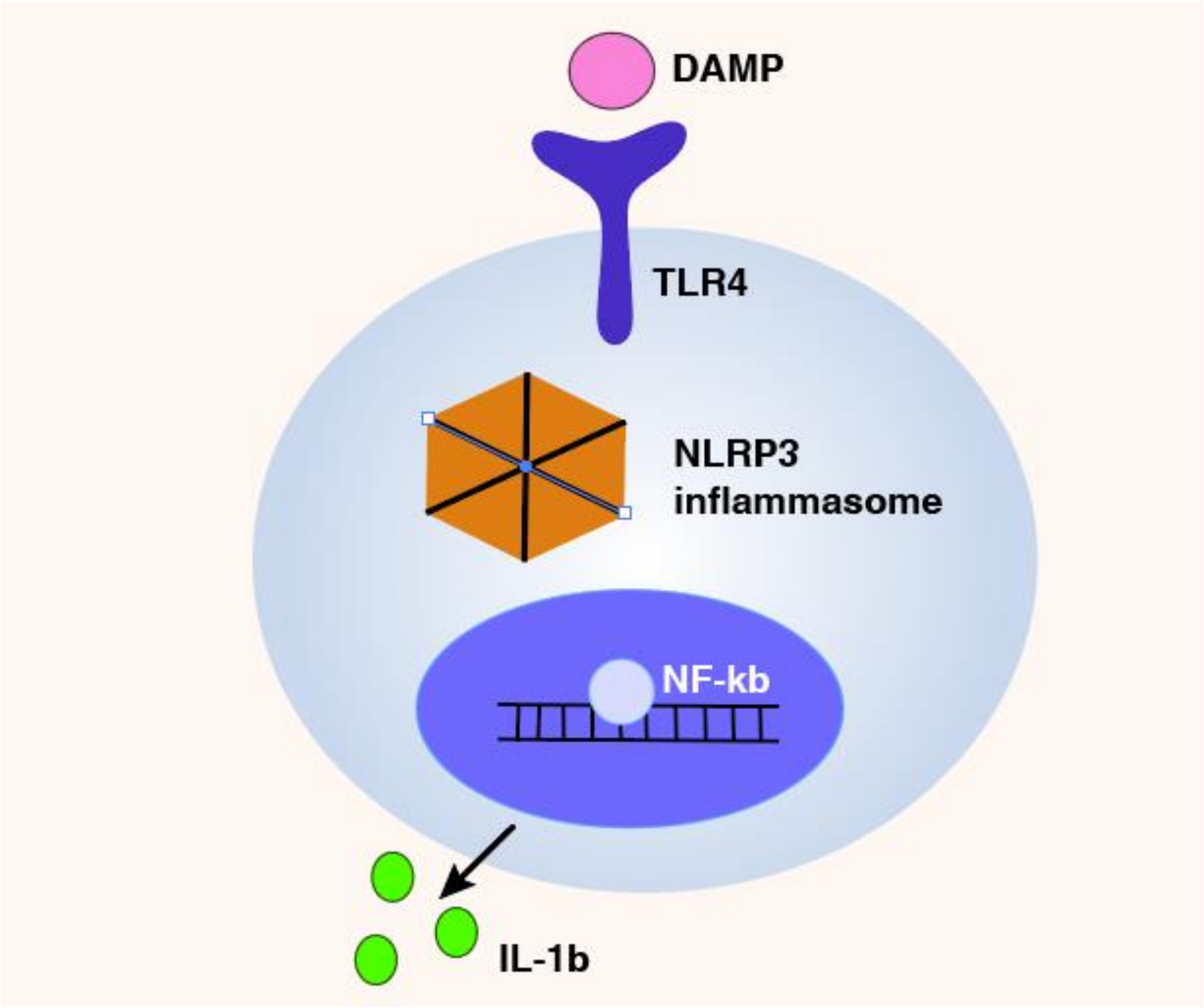
Heme and Immunothrombosis

- **Free heme** acts as a danger-associated molecular pattern (DAMP)

Activates **TLR4** on neutrophils, endothelium and platelet → cytokine release Amplifies complement activation (C3, C5b-9)

Induces endothelial adhesion molecules and tissue factor expression

Heme–NETs interaction amplifies immunothrombosis

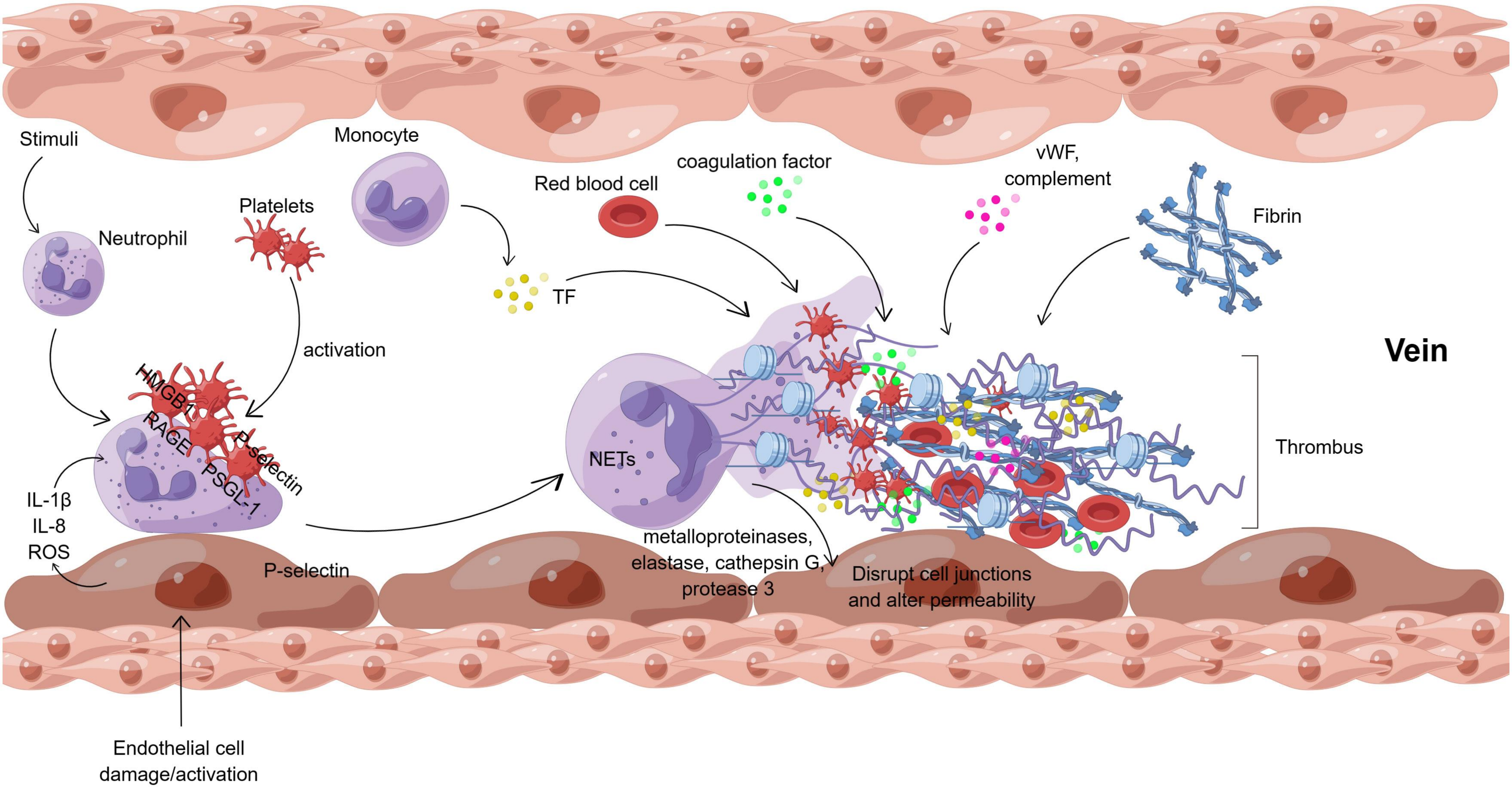

DAMP
TLR4
NLRP3
inflammasome
NF-kb
IL-1b

NETosis and Thromboinflammation

- NETs are web-like DNA structures released by activated neutrophils.

- Catalyzed by PAD4 enzyme and NADPH oxidase (NOX)-derived ROS.
- NETs activate platelets and coagulation factors.
- Inhibition of PAD4/NOX prolongs cerebral blood flow in sickle mice models.

Heparin and LMWH reduce NETosis and also block P-selectin.


Stimuli
Neutrophil
Platelets
Monocyte
Red blood cell
coagulation factor
vWF,
complement
Fibrin
TF
activation
Vein
HMGB1
RAGE
P-selectin
PSGL-1
NETs
Thrombus
IL-1β
IL-8
ROS
P-selectin
metalloproteinases,
elastase, cathepsin G,
protease 3
Disrupt cell junctions
and alter permeability
Endothelial cell
damage/activation

Role of Spleen and Splenectomy

- Spleen normally clears abnormal RBCs, PS-exposed cells, and microparticles
- **Splenectomy** → loss of this clearance system → persistence of procoagulant RBCs, MPs and thrombocytosis.

Strong association between splenectomy and portal vein thrombosis (PVT) in thalassemia.
Incidence of PVT post-splenectomy: 8–23%, depending on technique and spleen size (>1500 g).

Cerebral Thrombosis

- Prevalence: ~1.13% across 11,770 thalassemia patients.

Lesions include stroke, TIA, and silent cerebral infarct (SCI).

Risk factors: splenectomy (87% in stroke vs 63% in SCI), thrombocytosis, low protein C/S.

MRI recommended for early detection of subclinical lesions.

Biomarkers and Laboratory Indices for Thrombosis Monitoring in Hemoglobinopathies

	Representative Biomarkers
Hemolysis	LDH ↑, Reticulocyte count ↑, Free hemoglobin, Bilirubin ↑
Inflammation	IL-1β, IL-6, TNF-α, CRP, ferritin
Endothelial Activation / Damage	Soluble VCAM-1, E-selectin, P-selectin, von Willebrand factor (vWF)
Coagulation Abnormalities	D-dimer ↑, Fibrinogen ↑, Decreased Protein C/S, Low antithrombin III
Platelet Activation	β-thromboglobulin, soluble CD40L, P-selectin, platelet microparticles
Nitric Oxide / Oxidative Stress	L-Arginine ↓, Nitrite/Nitrate ↓, ROS ↑

Thrombosis in Pregnancy and Thalassemia

- Pregnancy increases hypercoagulability via hormonal and hemodynamic changes.
- *Reported cases*: β-thalassemia intermedia with portal vein thrombosis during pregnancy.
- *Mechanisms*: splenectomy, enlarged uterus compressing veins, and high platelet count.
- Anticoagulant prophylaxis and close monitoring recommended for pregnant thalassemia patients.

Therapeutic Approaches

Anticoagulation: LMWH → Warfarin → DOACs (case-specific).

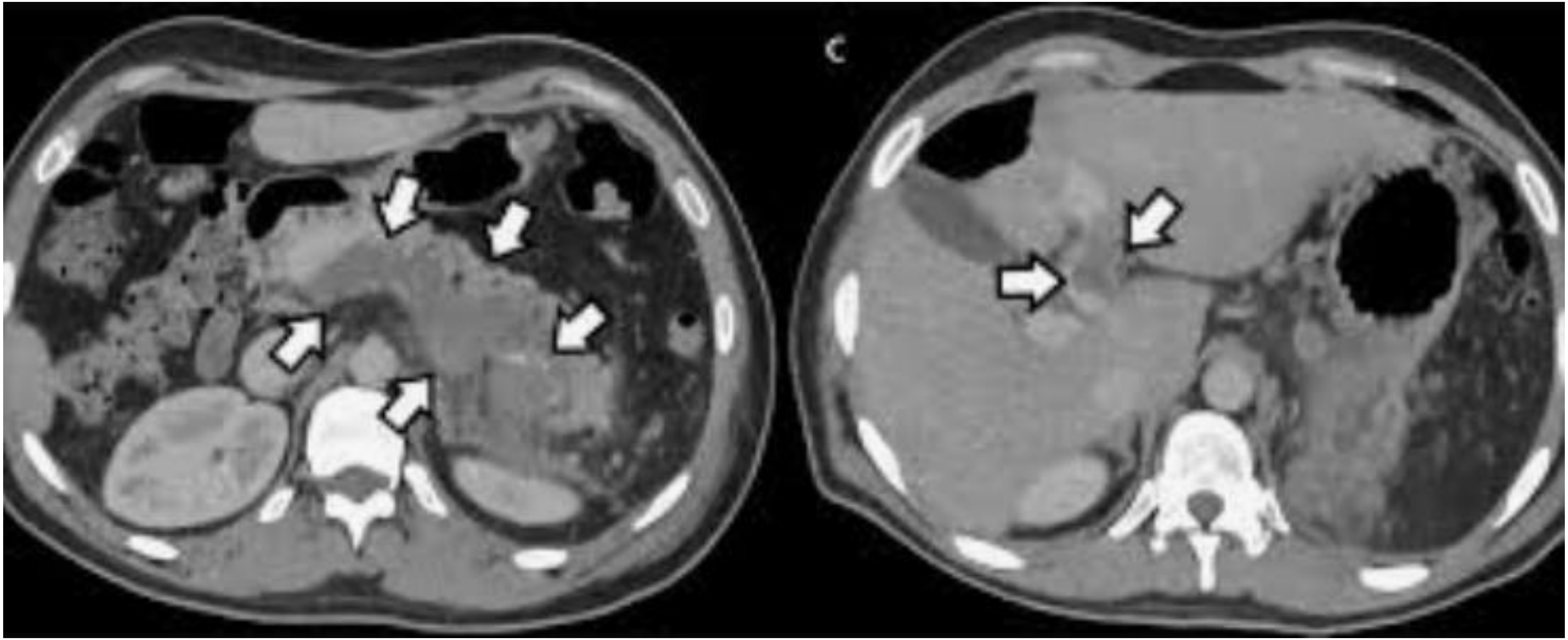
Hydroxyurea: decreases hemolysis, improves RBC deformability, reduces thrombosis risk.

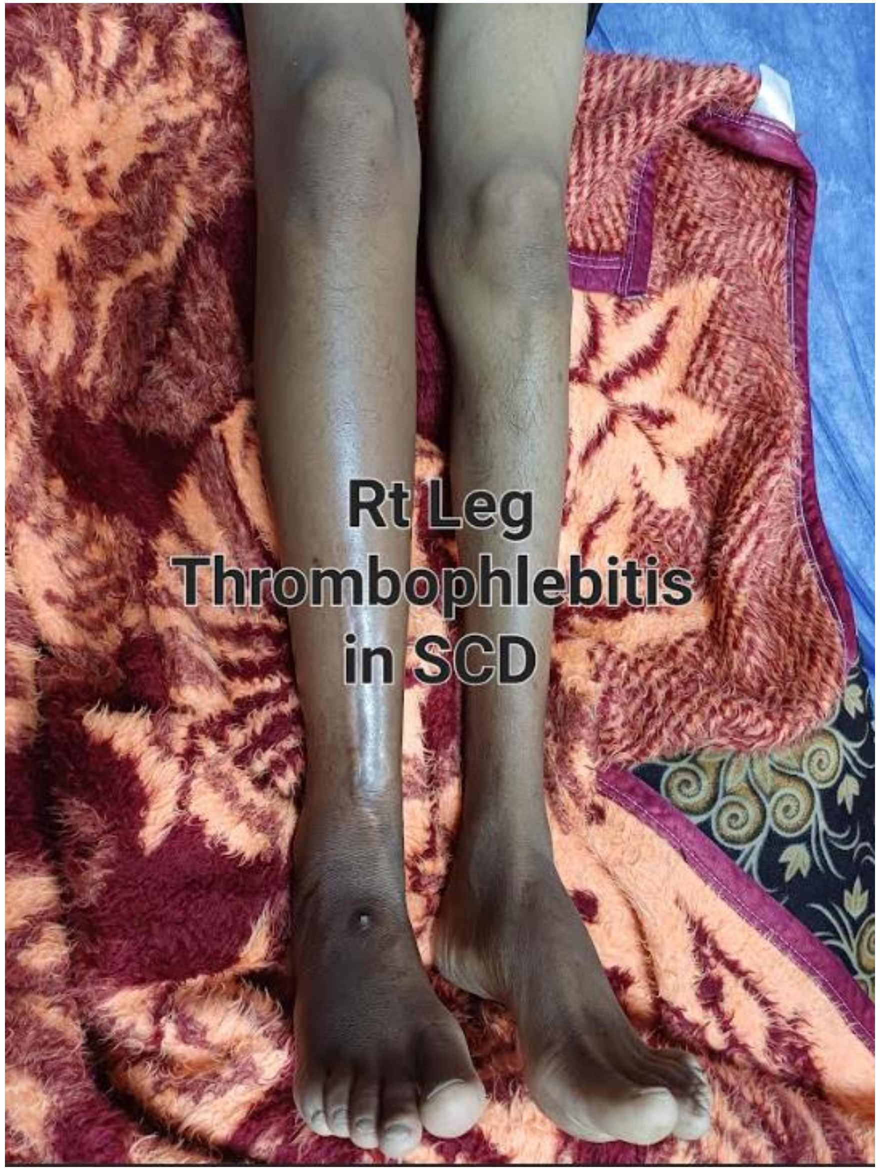
L-Arginine & NO donors: potential to improve vascular tone and reduce platelet activation.

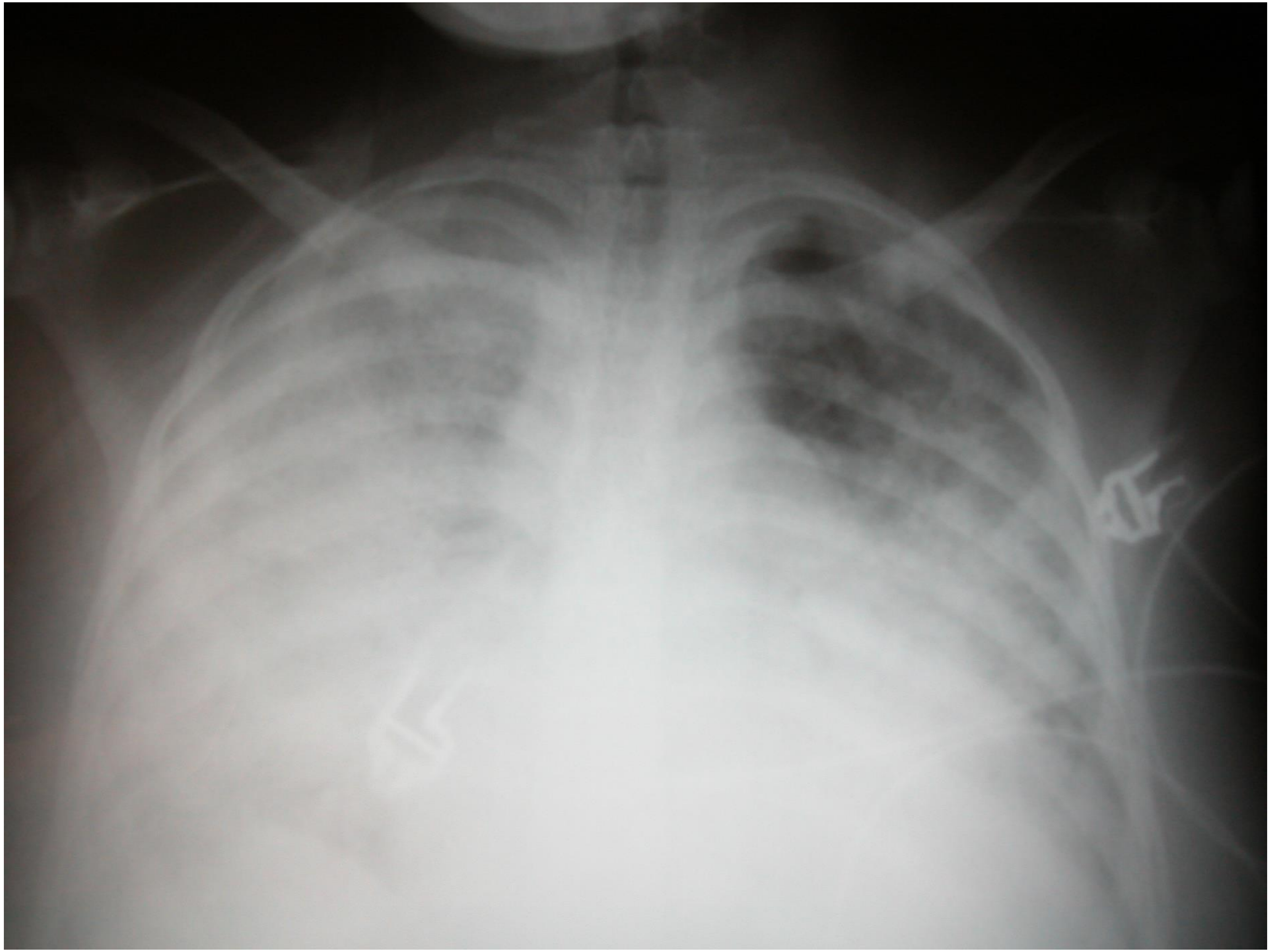
Transfusion therapy: prevents vaso-occlusive events but may cause iron overload and hyperviscosity.

Anticoagulant Prophylaxis in High-Risk Patients

- Thalassemia with splenectomy: *LMWH* → 4 weeks post-surgery.
- Consider long-term **prophylaxis** in recurrent PVT or cerebral thrombosis.
- Avoid aggressive anticoagulation if *platelet counts* $<65\times10^9/L$ or active bleeding.
- *Pregnancy*: individualized dosing based on coagulation profile.

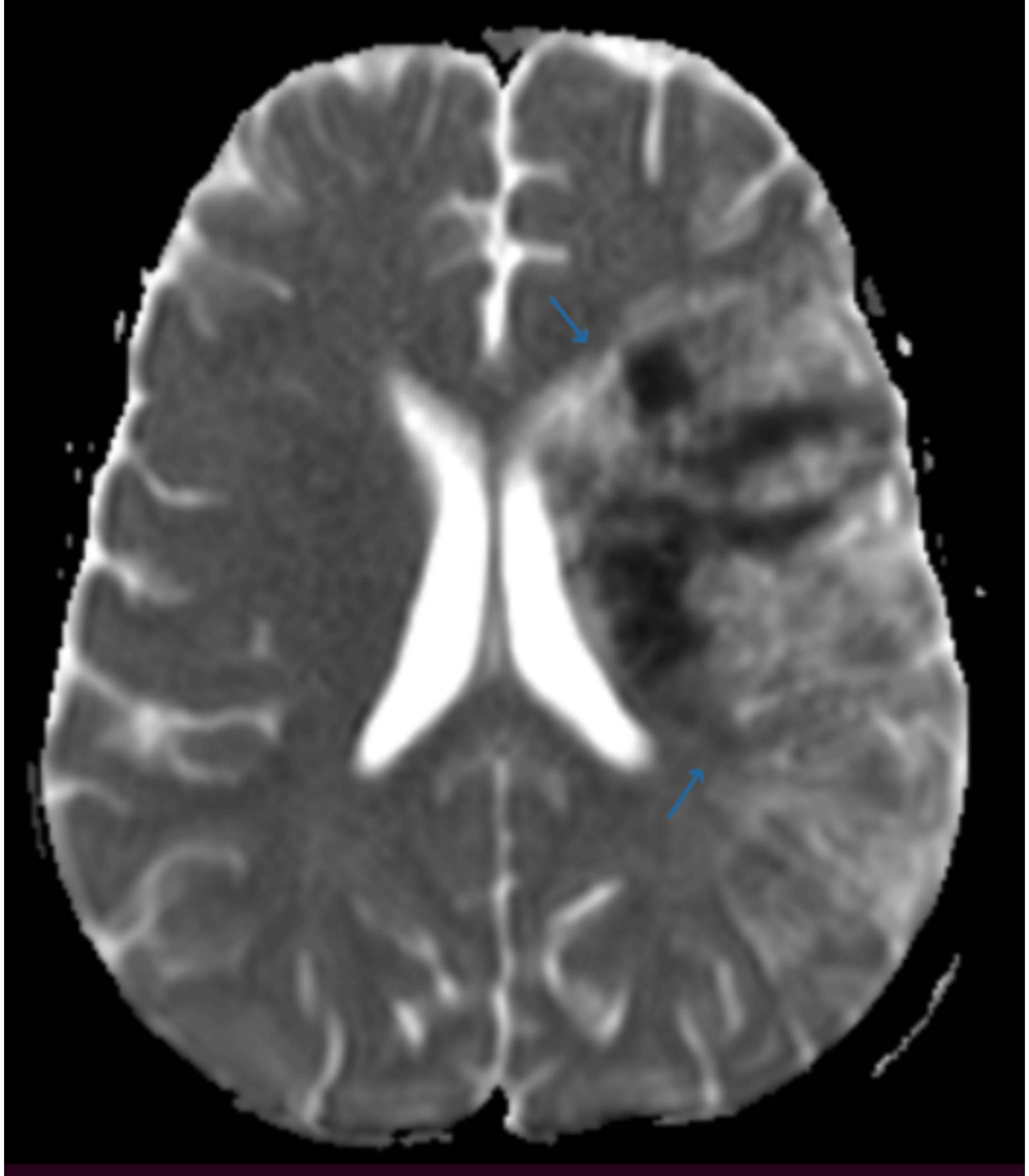

C

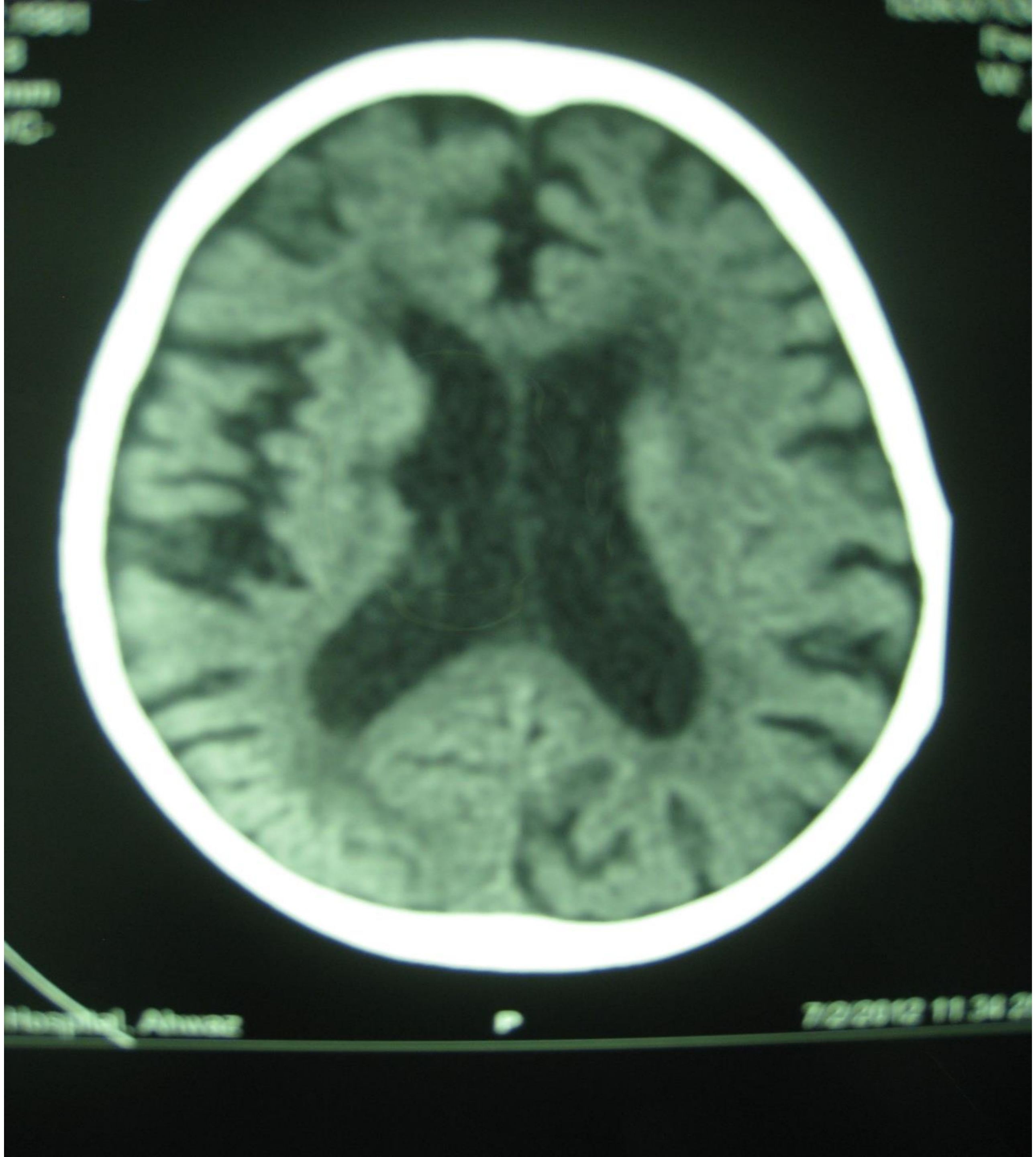

Rt Leg
Thrombophlebitis
in SCD




A

B

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Take away

- RBC abnormalities → PS exposure, microparticle release, and hemolysis.
- Hemolysis triggers inflammation and complement activation (HINT pathway).
- NETosis and splenectomy amplify thrombosis risk.
- Multi-organ involvement: brain, lungs, liver, kidneys (the role of plasmapheresis)
- Prevention requires integrated management: anticoagulation, anti-inflammatory, and disease-modifying therapy.