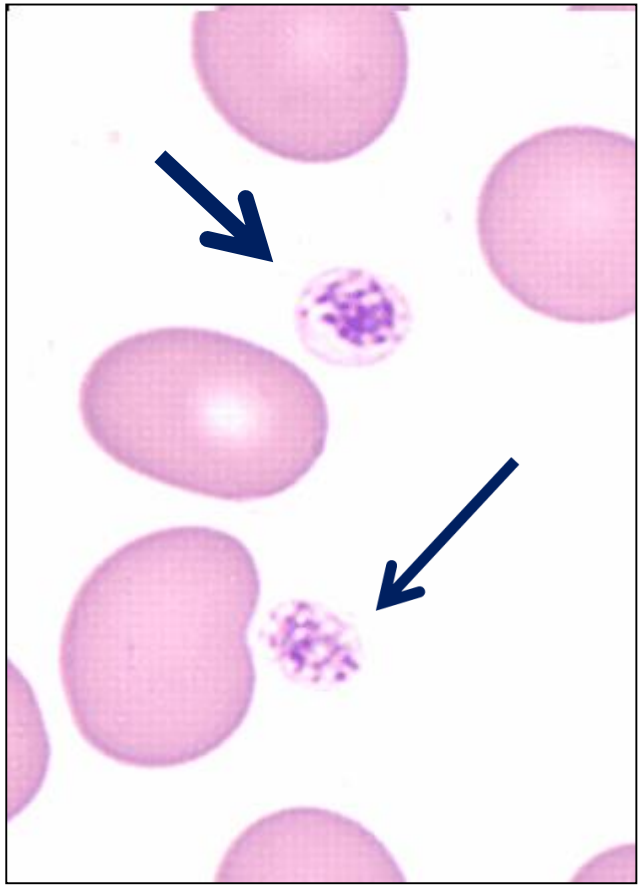
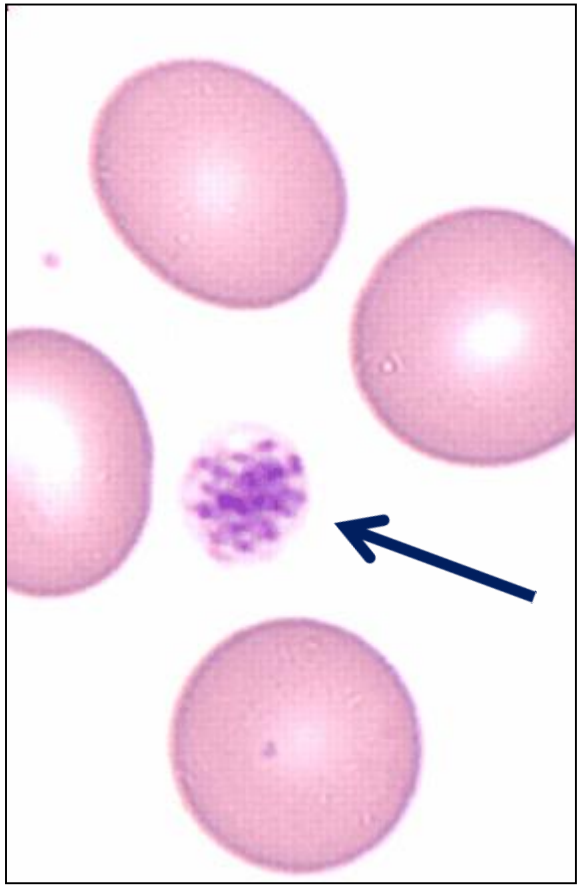
Piastrine (Prof. Gian Matteo Rigolin

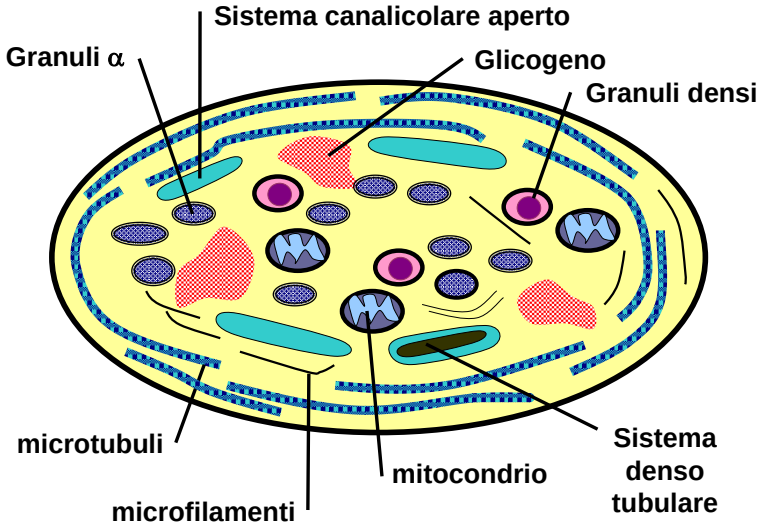




ASH Image Bank

piastrina

- Cellule anucleate che allo stato di riposo hanno forma discoidale, con asse maggiore di 2,9 μm, asse minore di 1,4 μm e volume medio di 6-7 μm3.
- La superficie liscia è delimitata dalla ***membrana piastrinica***, rivestita da ***glicocalice***, contenente glicocalicina, una frazione della glicoproteina Ib.
- Sotto la membrana, in connessione con l'estremità interna delle gp, si trova il ***citoscheletro***, costituito da ***microfilamenti*** e ***microtubuli***, strutture contrattili del sistema actina-miosina
- Il ***sistema canalicolare di connessione superficiale*** proveniente probabilmente da invaginazioni della membrana.
- Non associato alla membrana sembra invece il ***sistema tubulare denso***, coinvolto nel metabolismo dell'acido arachidonico.
- Il ***granulomero piastrinico***



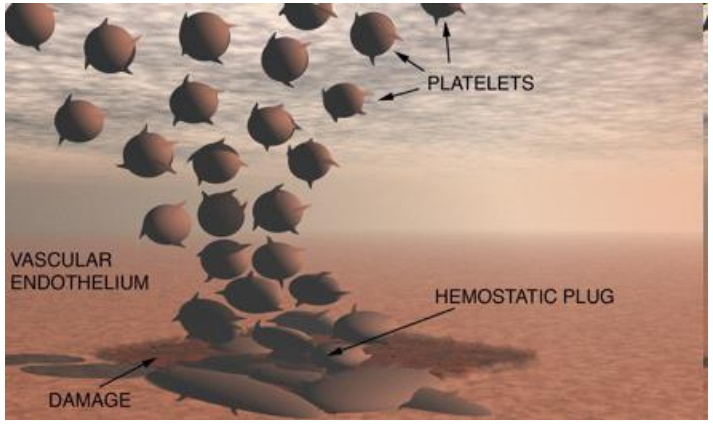
morfologia piastrinica: granuli

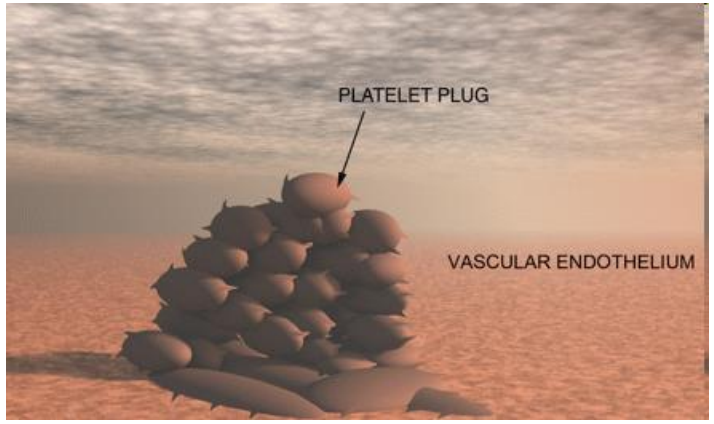
Granuli	Diametro (μm)	Contenuto
α-granuli	0,15-0,40	PF4, β-tromboglobulina Fibronectina, vitronectina, trombospondina Fibrinogeno, VWF, FV, FVIII, FXIII-a Inibitore del t-PA PDGF, PD-ECGF, TGF, HGF, EGF, CTAP-III
δ-granuli (corpi densi)	0,17	ADP, ATP, GTP, GDP Pirofosfato, ortofosfato Calcio-ioni, magnesio-ioni Serotonina, adrenalina, istamina
λ–granuli (lisosomi)	0,17-0,25	Fosfatasi acida, arilsolfatasi β-glucuronidasi, galattosidasi
Perossisomi	0,13-0,30	Catalasi
Granuli di glicogeno	0,015-0,030	Glicogeno (in ammassi)
Mitocondri	0,10-0,30	Enzimi del metabolismo energetico

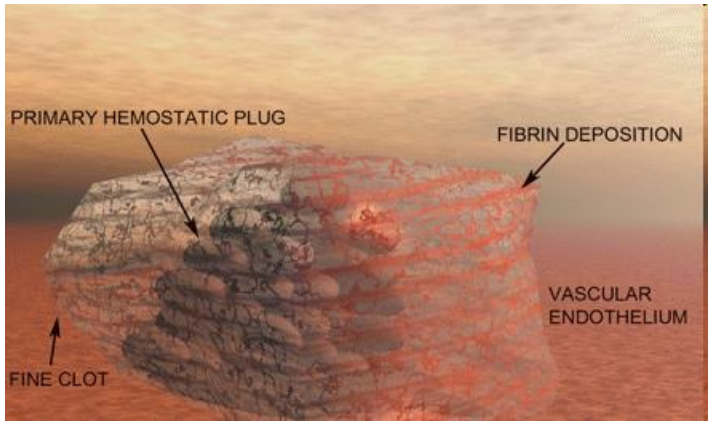
Funzioni delle piastrine

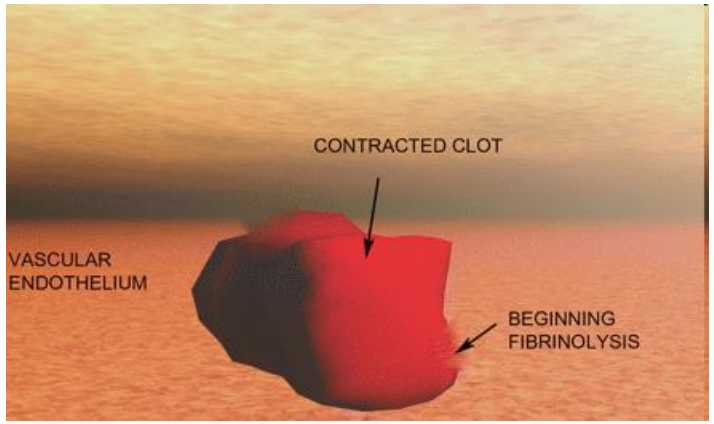
- **Adesione piastrinica**
- **Aggregazione piastrinica**
- **Attività procoagulante**
- **Stabilizzazione e retrazione del coagulo**
- **Fagocitosi**

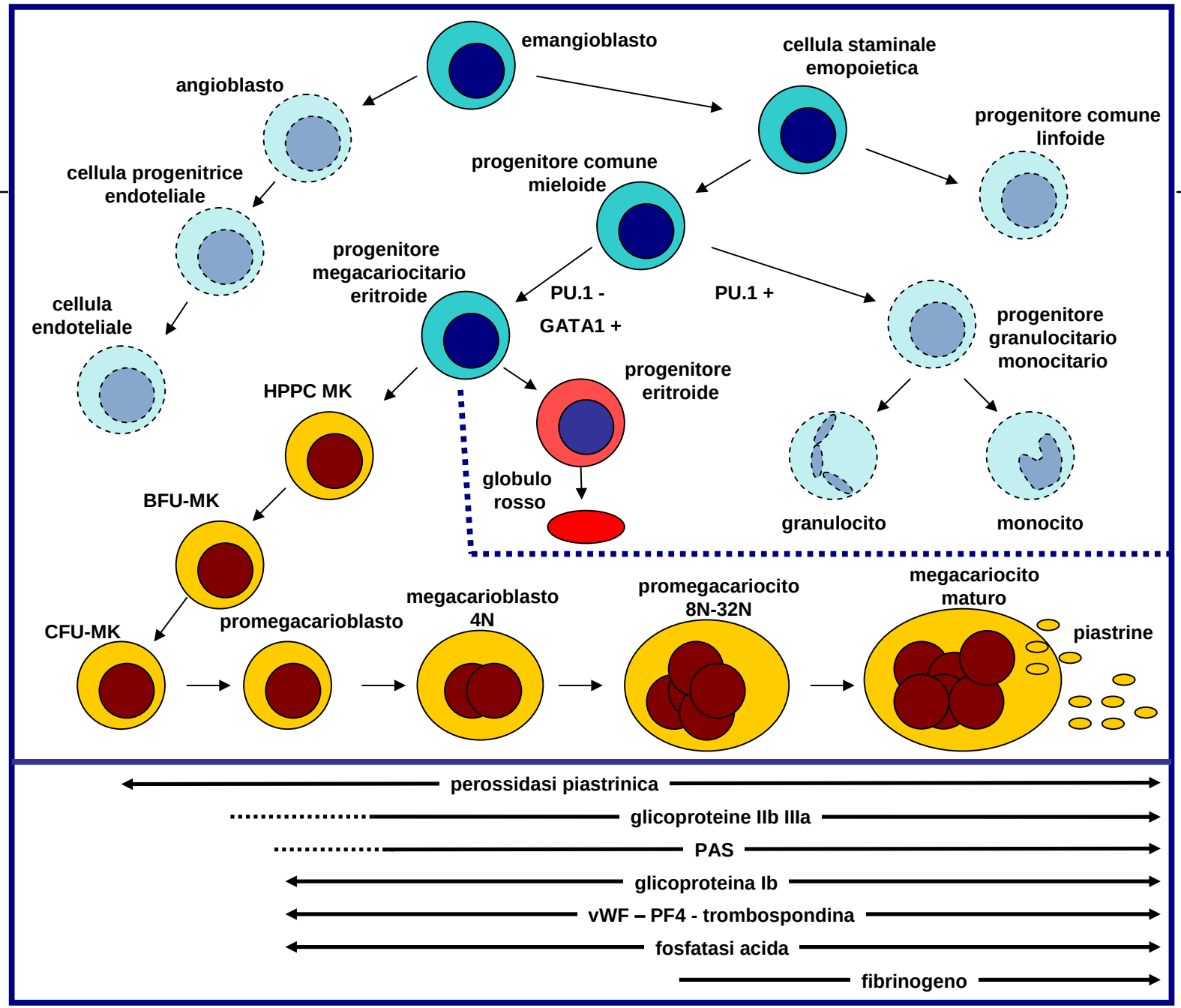
Funzioni delle piastrine



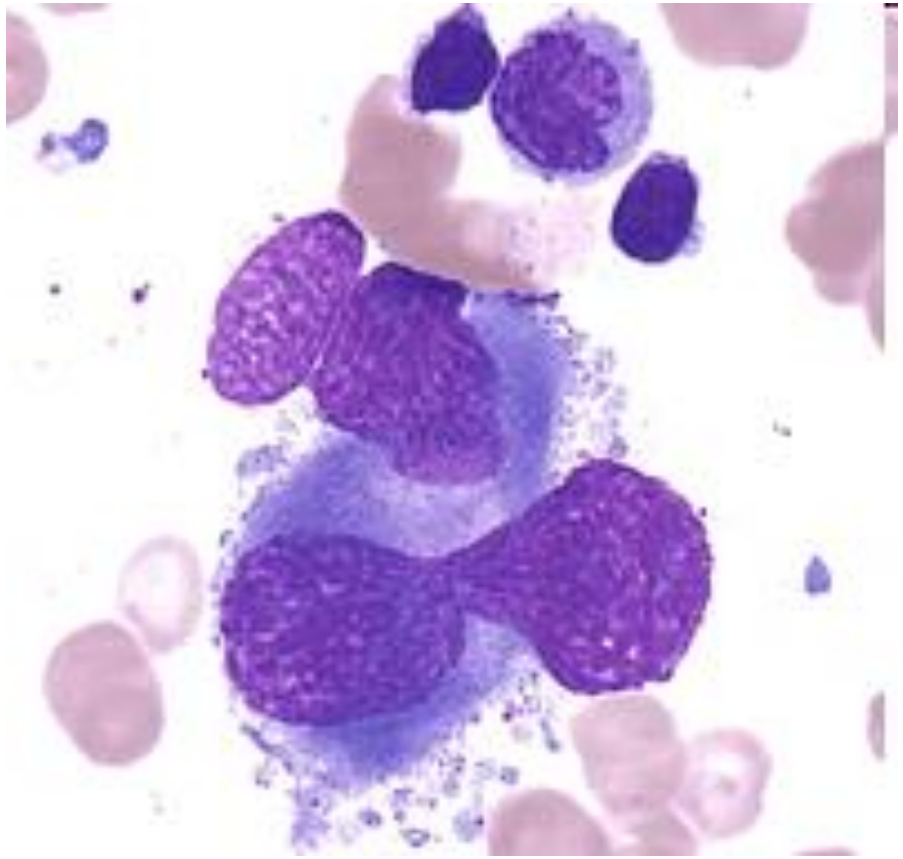







emangioblasto
angioblasto
cellula staminale emopoietica
progenitore comune linfoide
cellula progenitrice endoteliale
progenitore comune mieloide
progenitore megacariocitario eritroide
PU.1 -
GATA1 +
PU.1 +
cellula endoteliale
progenitore granulocitario monocitario
HPPC MK
progenitore eritroide
globulo rosso
granulocito
monocito
BFU-MK
CFU-MK
promegacarioblasto
megacarioblasto 4N
promegacariocito 8N-32N
megacariocito maturo
piastrine
perossidasi piastrinica
glicoproteine IIb IIIa
PAS
glicoproteina Ib
vWF – PF4 - trombospondina
fosfatasi acida
fibrinogeno

Formazione della piastrine



ASH Image Bank

Piastrinocinetica

- Il numero totale di piastrine circolanti, normalmente di 150 000-450 000/ul, è il risultato del rapporto tra piastrine prodotte a livello midollare e piastrine distrutte in sede periferica.

- È stato calcolato che la piastrinopoiesi midollare normale produce circa 35 000-50 000 piastrine/ul di sangue al giorno (**turn over piastrinico giornaliero**), ma questo dato può aumentare notevolmente quando la megacariocitopoiesi viene stimolata dalla deplezione piastrinica periferica acuta o cronica: il meccanismo di feedback positivo è mediato dall'intervento dei fattori umorali regolatori e stimolatori della megacariocito- e piastrinopoiesi.

Piastrinocinetica

- In circolo, le piastrine più giovani sono più grandi (aumentato volume piastrinico medio o MPV) e contengono RNA: queste piastrine vengono pertanto chiamate piastrine "reticolate" in quanto ritenute analoghe ai reticolociti.

- Queste caratteristiche possono essere specificamente studiate dai moderni emocitometri automatizzati anche se il significato clinico di tale determinazione è ancora non ben definito.

Piastrinocinetica

- È stato suggerito che la determinazione delle piastrine reticolate (valore normale 1,3%, range 1,1-1,5%) possa consentire una discriminazione tra le piastrinopenie
  - da “normale o ridotta trombocitopoiesi” valore medio di piastrine reticolate 7,5%, range 5,3-9,7%) rispetto a quelle
  - da “aumentato turnover piastrinico” (valore medio 30%, range 25-35%).

piastrinocinetica

- La ***vita media piastrinica*** nel normale è di circa 8-10 giorni e diminuisce nelle piastrinopenie da consumo.

- Dalla determinazione del tempo di sopravvivenza in circolo delle piastrine si può dedurre il ***tricambio*** (turn over ***piastrinico giornaliero***), che nel normale è di circa 40.000 piastrine per ul.

piastrinocinetica

- Le piastrine immesse in circolo, sia direttamente dal midollo sia attraverso trasfusione, si distribuiscono in due compartimenti, **quello plasmatico e quello splenico**.

- Quest'ultimo rappresenta nel normale circa un terzo del totale, è proporzionale alla massa splenica, aumentando nelle splenomegalie, e non determina la distruzione delle piastrine sequestrate, che possono essere rimesse in circolo in libero scambio con il *pool* plasmatico.

piastrinocinetica

- La ***piastrinocateresi*** avviene a livello del SRE, soprattutto splenico, ma anche epatico, e in piccola parte forse nel midollo osseo e nei linfonodi.

Normalmente il rapporto tra cateresi splenica ed epatica è di 3:1, ma considerando le dimensioni dei due organi si deduce che l'attività splenica è circa 20 volte superiore a quella epatica.

Classificazione patogenetica delle piastrinopenie

1. Da deficiente piastrinopoiesi
2. Da esaltata distruzione o aumentato consumo periferico
3. Da alterata distribuzione della massa piastrinica
4. Falsa piastrinopenia

1. Da deficiente piastrinopoiesi

1. Da ipo- o aplasia megacariocitaria midollare
   1. *Congenita ereditaria*, WAS
   2. *Congenita non ereditaria*: infezioni, farmaci
   3. *Acquisita*: infezioni, farmaci, radiazioni, invasione o sostituzione midollare da emopatie o tumori metastatici

2. Da piastrinopoiesi inefficace
   1. Carenze vitaminiche (vit. B_{12}, acido folico)
   2. Sindromi mielodisplastiche

3. Da alterazioni dei fattori di controllo delle piastrinopoiesi
   1. Carenza di trombopoietina o di altri fattori stimolanti la piastrinopoiesi
   2. Da instabile regolazione della piastrinopoiesi

2. Da esaltata distruzione o aumentato consumo periferico

I. Per meccanismo immunologico
   A. Da autoanticorpi
      i. PPI (Morbo di Werlhof)
      ii. Associata a malattie autoimmuni
      iii. Sindrome da anticorpi antifosfolipidi
   B. Da alloanticorpi
      i. piastrinopenia alloimmune del neonato
      ii. porpora post-trasfusionale
      iii. refrattarietà alle trasfusioni piastriniche
   C. Da farmaci

II. Per meccanismo non immunologico
   A. Piastrinopenia non immunologia acquisita (CID)
   B. Processi microangiopatici (TTP e HUS)
   C. Piastrinopenia non immunologica del neonato
   D. Sindrome di Kasabach-Merritt
   E. Da circolazione extracorporea
   F. Da infezioni batteriche, virali, protozoarie

3. Da alterata distribuzione della massa piastrinica

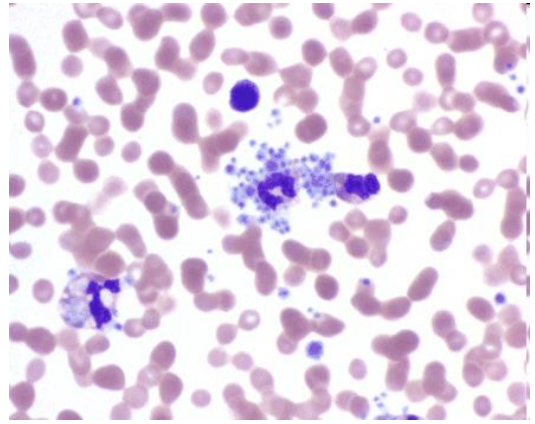
I. Da sequestro
   A. Ipersplenismo, splenomegalie
   B. Emangiomatosi diffusa

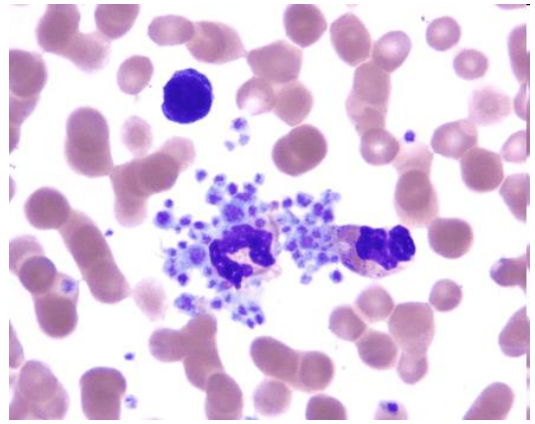
I. Diluizione: da trasfusioni plasmatiche

II. Anestesia ipotermica

Falsa piastrinopenia

- Pseudopiastrinopenia da EDTA
  - causata da atc anti-pst.
  - In condizioni normali (in vivo) questi atc non causano agglutinazione perché i relativi antigeni piastrinici restano “nascosti” all’interno della pst. Il contatto delle pst con l’EDTA della provetta, causa una modifica della struttura piastrinica con conseguente “scopertura” degli antigeni nascosti che si rendono disponibili per il legame con l’atc: si realizza così l’agglutinazione delle pst che sono sottratte al conteggio





Porpora trombocitopenica immune Malattia di Werlhof

- **disordine autoimmunitario, caratterizzato da piastrinopenia e emorragie mucoso-cutanee.**

- **incidenza 10 casi su 100.000 persone/anno**
- **è la più frequente tra le malattie emorragiche (20-25%).**

- **può essere acuta (durata <6 mesi) o cronica**
- **Può essere:**
  - **primitiva**
  - **secondaria**
    - **Da farmaci.**
    - **LES,**
    - **sindrome da anticorpi antifosfolipidi,**
    - **immunodeficienze (deficit di IgA, ipogammaglobulinemia comune variabile),**
    - **disordini linfoproliferativi (LLC, LGL, linfomi),**
    - **infezioni (HIV, HCV, CMV, H. Pylori, HZV)**
    - **Dopo vaccinazioni**
    - **Post trapianto di cellule staminali**

Primary ITP

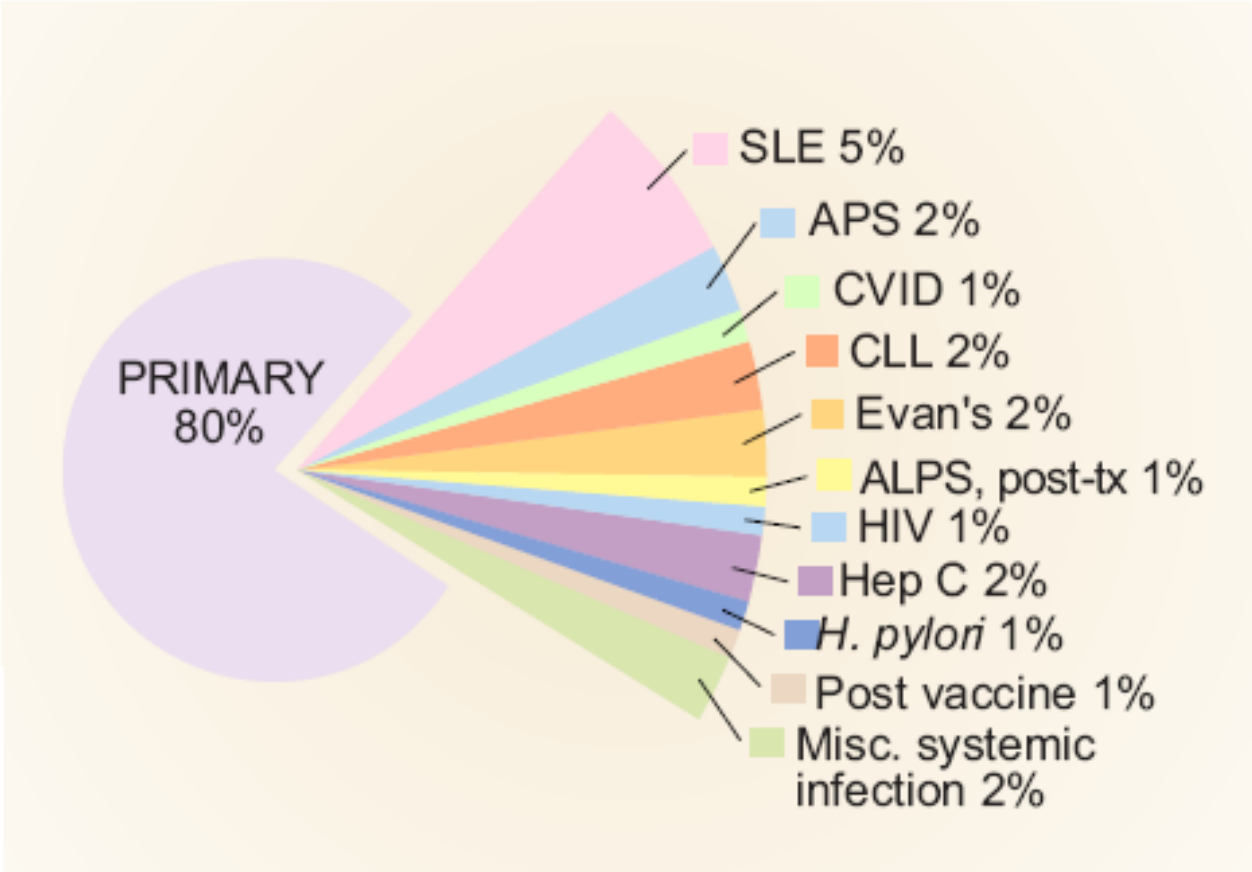
- **Primary ITP is an autoimmune disorder characterized by isolated thrombocytopenia (PB Plt<100 x 10^9/L) in the absence of other causes or disorders that may be associated with thrombocytopenia.**

- **The diagnosis of primary ITP remains one of exclusion;**
  - **no robust clinical or laboratory parameters are currently available to establish its diagnosis with accuracy.**

Blood. 2009;113:2386-2393

Secondary ITP

- **All forms of immune-mediated thrombocytopenia except primary ITP**



Blood. 2009;113:6511-6521

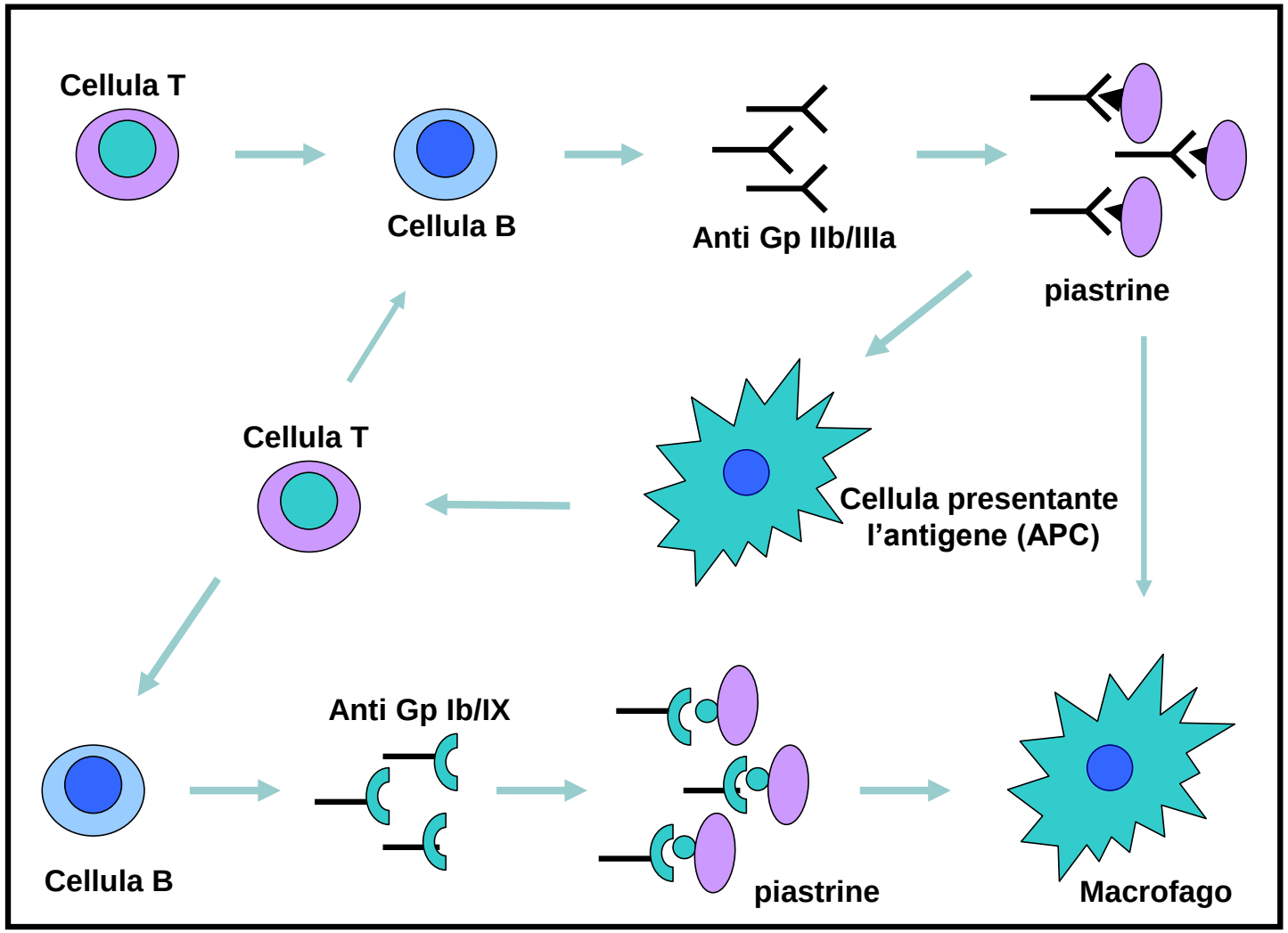
Blood. 2009;113:2386-2393

Phases of the disease

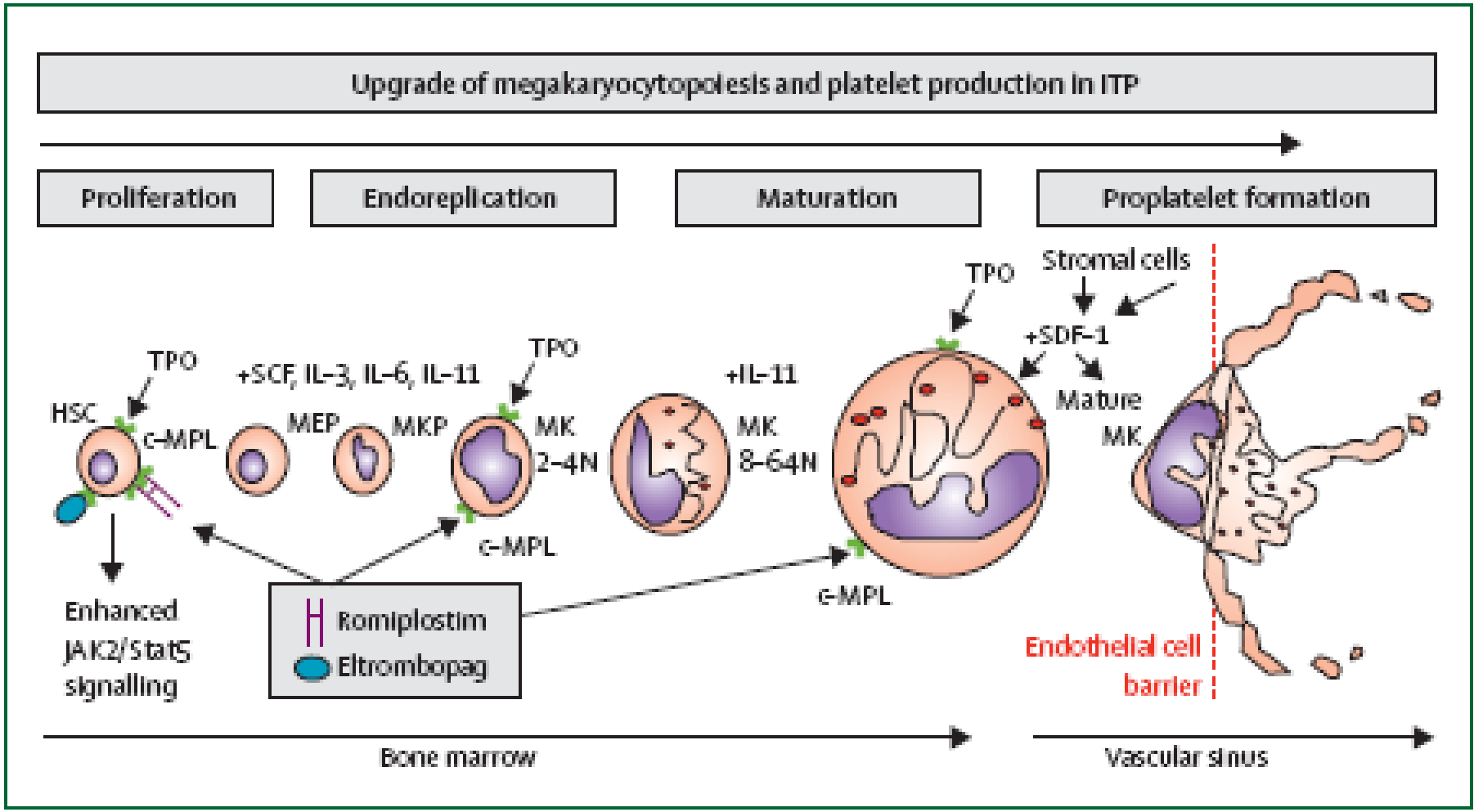
- **Newly diagnosed ITP:**
  - **within 3 months from diagnosis**
- **Persistent ITP:**
  - **between 3 to 12 months from diagnosis.**
  - **Includes patients not reaching spontaneous remission or not maintaining complete response off therapy.**
- **Chronic ITP:**
  - **lasting for more than 12 months**
- **Severe ITP:**
  - **Presence of bleeding symptoms at presentation sufficient to mandate treatment, or**
  - **occurrence of new bleeding symptoms requiring additional therapeutic intervention with a different plt-enhancing agent or an increased dose**

Blood. 2009;113:2386-2393

eziopatogenesi



New-generation drugs that stimulate platelet production in chronic ITP



Lancet 2009;373:1562

eziopatogenesi

1. vi sono dati che suggeriscono la presenza anche di una **piastrinopoiesi inefficace** con livelli solo moderatamente aumentati di TPO.

2. **l'Helicobacter pylori**, un batterio Gram-positivo coinvolto nella patogenesi della gastrite, dell'ulcera gastrica e dei linfomi gastrici tipo MALT, possa essere implicato nei meccanismi che portano all'insorgenza di vari disordini di tipo autoimmunitario tra i quali viene annoverata anche la PPI.

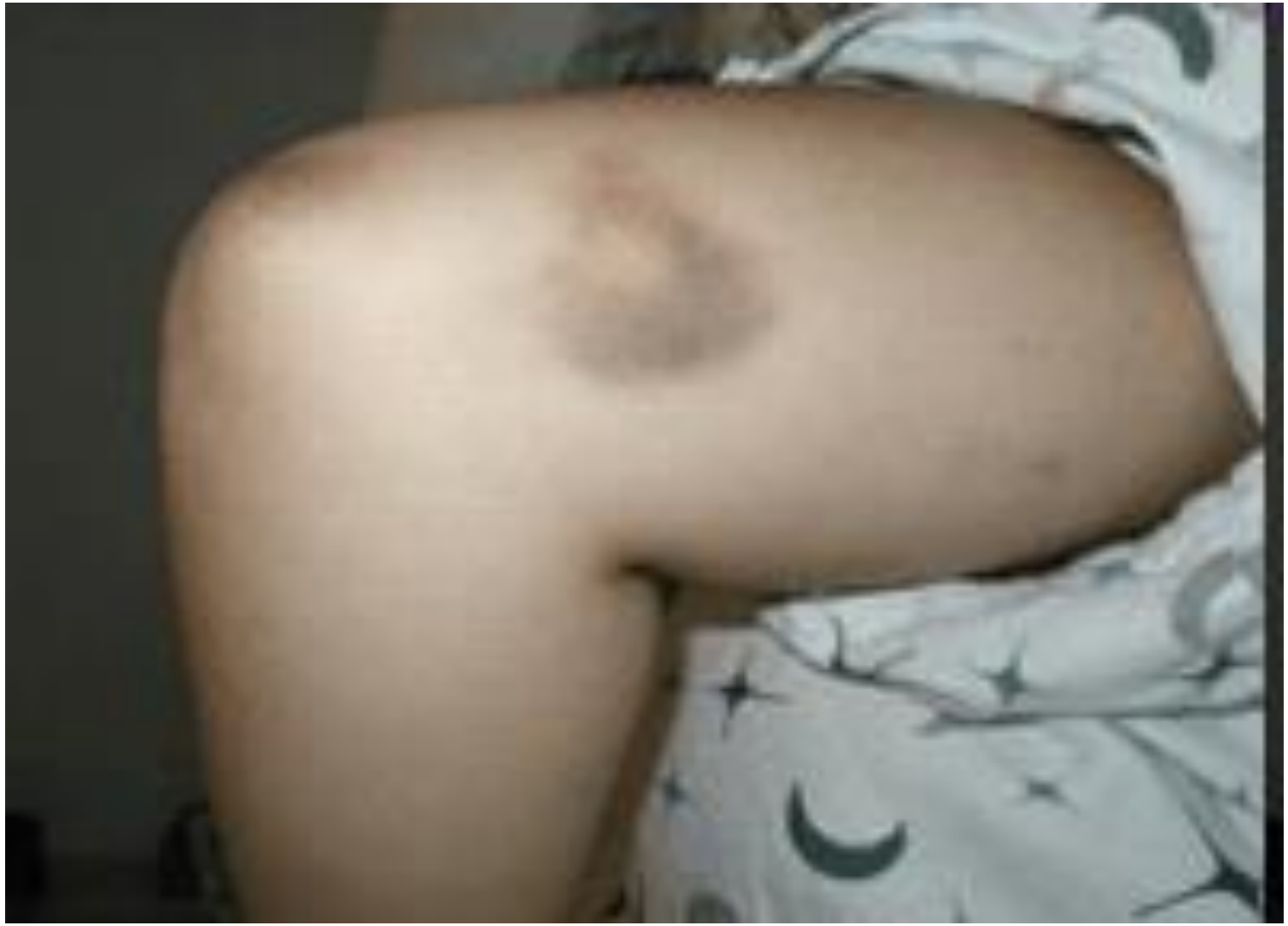
sintomi

- È caratterizzata dalle manifestazioni emorragiche, che possono essere gravi e diffuse a tutto l'ambito cutaneo, alle mucose e agli organi interni, ma possono anche essere discrete e localizzate.

- l'esordio può essere
  - insidioso e progressivo,
  - ma più spesso acuto, talora in occasione di piccoli interventi chirurgici (estrazioni dentarie, tonsillectomia, tonsillectomia) o di episodi infettivi intercorrenti

Manifestazioni emorragiche all'esordio della PPI

Sintomi emorragici	**Percentuale**
Porpora cutanea	70-80
Emorragie mucose	20-30
Emorragie uterine	10-15
Emorragie retiniche	1-4
Ematuria	1-2
Emorragie cerebromeningee	0,5-1



Confronto tra forma acuta e forma cronica delle PPI

	Forma acuta	**Forma cronica**
Età di incidenza massima	2-6 anni	20-40 anni
M:F	1:1	1:4
Incidenza stagionale	Primavera-autunno	Indifferente
Infezioni pregresse	Frequenti	Non frequenti
Tasso piastrinico	$<30 \times 10^9/l$	$30\text{-}130 \times 10^9/l$
Insorgenza dei sintomi	Improvvisa	Graduale
Emorragie mucose	Frequenti	Infrequenti
Durata media	2-6 settimane	Anni
Remissione spontanea	>80% dei casi	Rara
Decorso	*Self-limited*	*Self-perpetuating*
Prognosi	Favorevole	Riservata

PPI e gravidanza

- Gravidanza: 1-2 casi ogni 1000 gravidanze, e che rappresenti il 3% di tutte le cause di piastrinopenia al momento del parto.

- La diagnosi differenziale include
  - la sindrome HELLP (hypertension, elevated liver enzymes and low platelet count),
  - processi emolitici microangiopatici,
  - le trombocitopenie ereditarie
  - la trombocitopenia gestazionale da emodiluizione (o benigna) 75% dei casi di piastrinopenia in gravidanza.

TABLE 3 • Differential diagnosis of thrombocytopenias in pregnancy.

Cause of thrombocytopenia	Time of the most common onset	Grade of thrombocytopenia	Biochemical abnormalities	Clinical symptoms
Gestational	III trimester	mild	no	no
ITP	I-II trimester	mild to severe	no	bleeding in severe cases
Eclampsia	III trimester	mild to severe	DIC[4] proteinuria	hypertension
HELLP[1]	III trimester	mild to severe	DIC, hemolytic anemia ↑AST/ALT	no or complex presentation
TTP[2]	II trimester	mild	hemolytic anemia	fever, CNS[5]
HUS[3]	Post - partum	mild	hemolytic anemia ↑creatinine	fever, renal failure
AFL[6]	III trimester	mild	DIC, hemolytic anemia, hypoglycemia	complex presentation

[1]Hemolytic anemia, elevated liver enzyme, low platelet count; [2]thrombotic thrombocytopenic purpura; [3]Hemolytic uremic syndrome; [4]disseminated intravascular coagulation; [5]involvement of central nervous system; [6]acute fatty liver.

Rodeghiero DCTH 2015

Altre forme cliniche

- **La PPI neonatale** da madre affetta da PPI insorge a causa del passaggio transplacentare degli atc antiplt materni.
  - Il 10% dei neonati presenta alla nascita e nei primi giorni di vita livelli piastrinici inferiori a 50 x 10^9/l, ma solo nel 4% dei casi le piastrine scendono al di sotto di 20 x 10^9/l.
  - Il rischio di complicanze emorragiche intracraniche è <dell'1% e non aumentato dal parto cesareo.

Altre forme cliniche

- La **piastrinopenia HIV-correlata** nella maggior parte dei casi è di grado lieve e clinicamente muta.
  - La sua patogenesi è in parte correlabile con quella della PPI in parte legata ad un’azione diretta del virus a livello midollare.

Altre forme cliniche

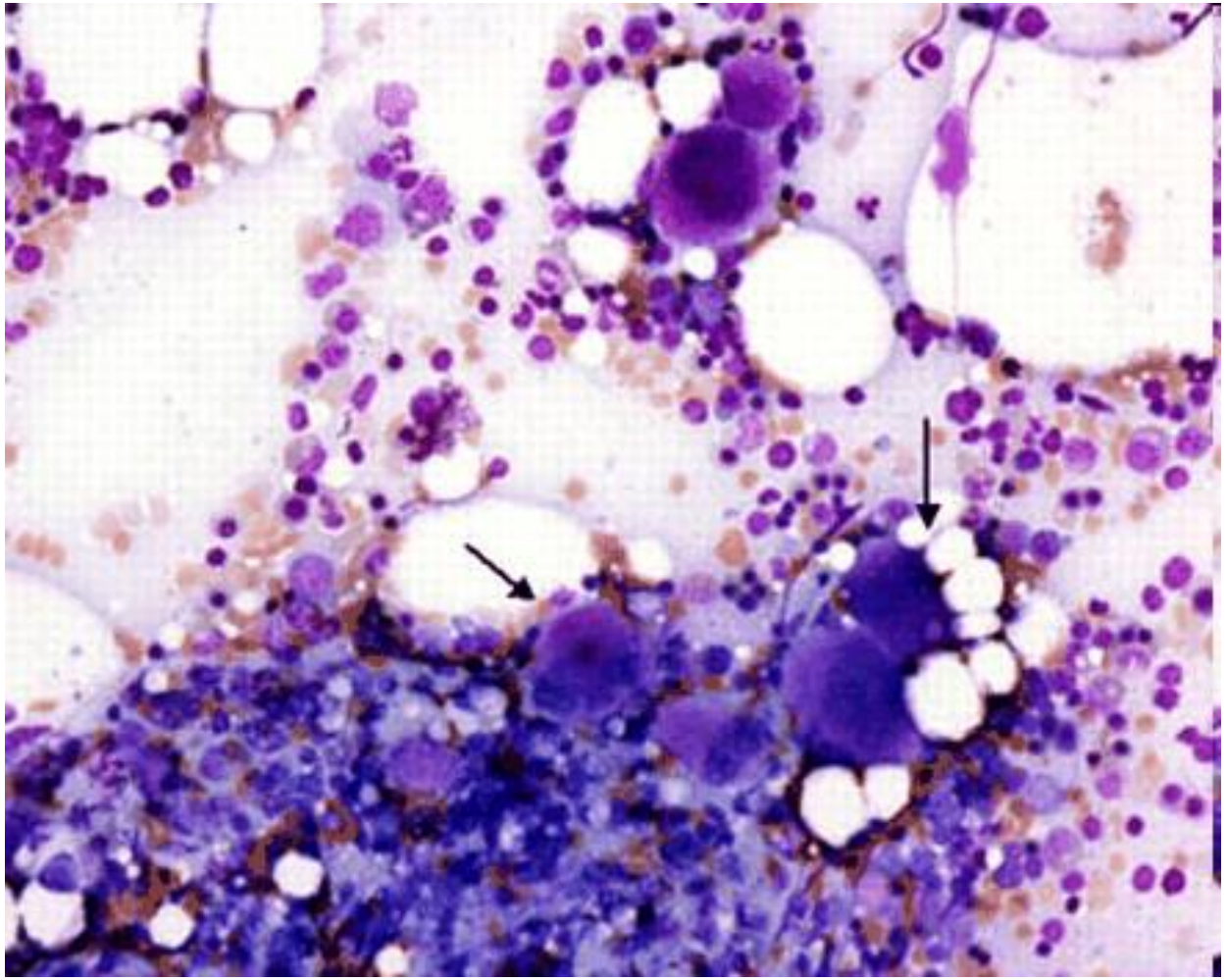
- La **piastrinopenia della sindrome da anticorpi antifosfolipidi (APS),** ha come causa la presenza di atc anti-plt, con specificità diversa (antiglicoproteina Ib/IX, IIb/IIIa, IV) rispetto a quella degli atc antifosfolipidi.

  - La piastrinopenia non riduce il rischio di complicanze trombotiche, se non per valori inferiori a 50 x 10^9/l quando può favorire la comparsa di complicanze emorragiche se associata a trattamenti anticoagulanti.

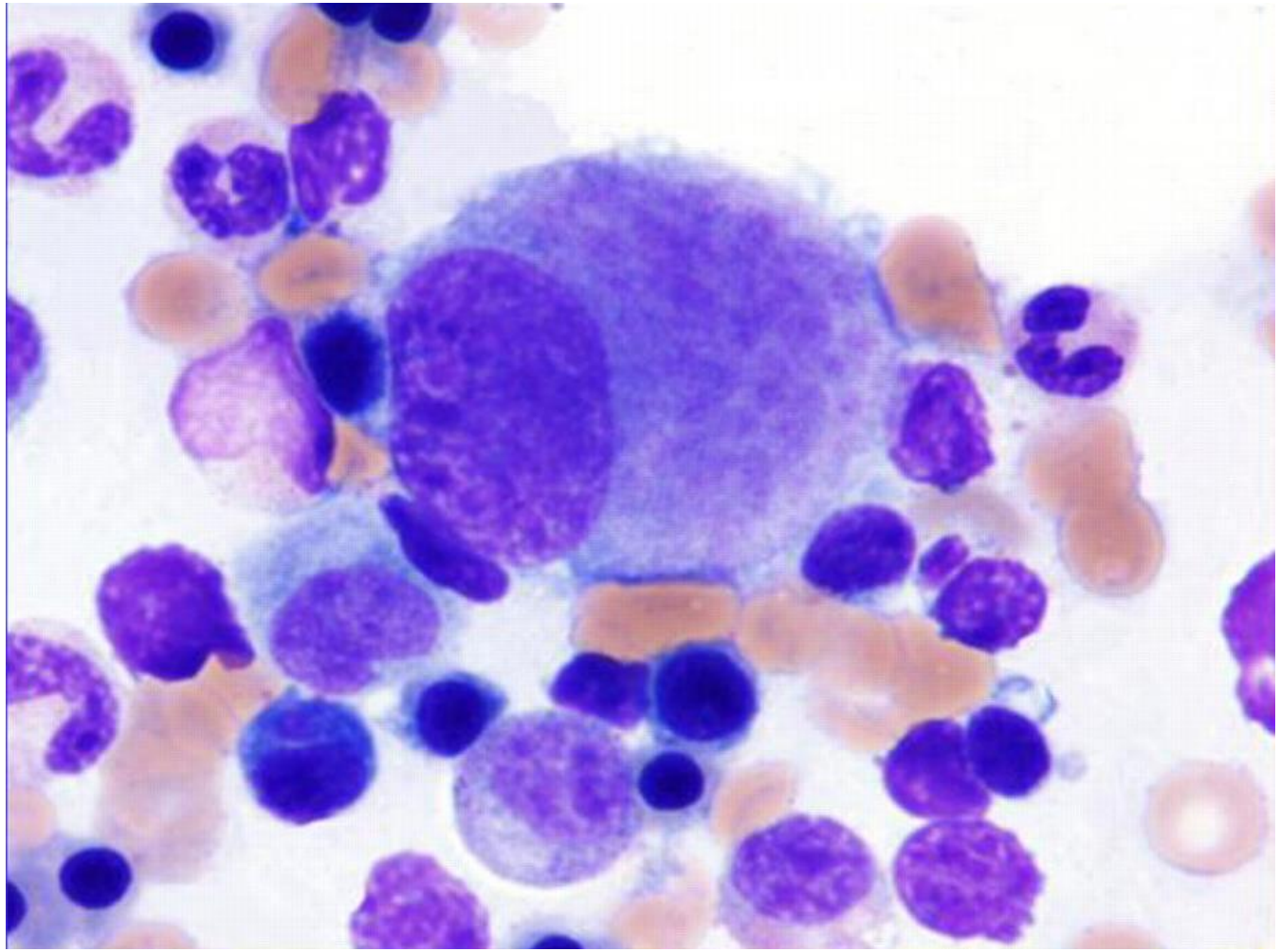
diagnosi

- **Piastrinopenia**
- **Nella norma i test emocoagulatori (PT e APTT), e il dosaggio del fibrinogeno e degli altri fattori plasmatici**
- **Iperplasia megacariocitaria**

PPI: iperplasia megacariocitaria



PPI: iperplasia megacariocitaria



Utility of various evaluations in the diagnosis of ITP

Basic evaluation	Tests of potential utility	Tests of unproven benefit
Patient/family history	Glycoprotein-specific antibody	Thrombopoietin
Physical examination	Antiphospholipid antibodies (including anticardiolipin and lupus anticoagulant)	Reticulated platelets
Complete blood count and reticulocyte count	Antithyroid antibodies and thyroid function	Platelet-associated immunoglobulin G
Peripheral blood film	Pregnancy test in women of childbearing potential	Bleeding time
Quantitative immunoglobulin level measurement*	Antinuclear antibodies	Platelet survival study
Bone marrow examination (in selected patients)	Viral PCR for parvovirus and CMV	Serum complement
Blood group (Rh)		
Direct antiglobulin test		
H pylori†		
HIV†		
HCV†		

Adapted from Provan et al.[4]

CMV, cytomegalovirus; HCV, hepatitis C virus; PCR, polymerase chain reaction; Rh, rhesus.

*Quantitative immunoglobulin level measurement should be considered in children with ITP and is recommended in those children with persistent or chronic ITP as part of reassessment evaluation.

†Recommended by the majority of the panel for adult patients regardless of geographic location.

Blood 2010;115:168

Lambert P. Blood. 2017;129(21):2829-2835

Laboratory test for ITP

THERE IS NO BIOMARKER OR GOLD STANDARD TEST

A MORE ACCURATE DIAGNOSIS OF ITP
SHOULD RELY
BOTH ON CLINICAL AND LABORATORY INDICATORS

Kelton JG et al. Hematology ASH 2018

Suggested approach to the evaluation of a patients with suspetcted ITP

- **The history and the physical examination**
  - **focused on**
    - **estimating the duration of the thrombocytopenia and**
    - **the exclusion of other thromboctytopenic and secondary imune thrombocytpenic disorders**
  - **Evaluation of**
    - **Hemostatic impairment**
    - **Precipitating causes (infections, alchol, among the others)**
    - **Physical examination for evidence of bleeding, adenopathies, hepatosplenomegaly (abdominal ultrasound may help)**

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Suggested approach to the evaluation of a patients with suspetcted ITP

- LABORATORY TESTS
  - Antinulear antibody (ANA)
  - Anticardiolipin antibody (ACA)
  - Quantitative immunoglobulins
  - Serum electrophoresis
  - Hepatitis B and C screening
  - Thyrotropin
- RATIONALE
  - ANA, ACA for secondary ITP associated with SLE or antiphospholipid syndrome (risk of thrombosis if TPA-RA are used!)
    - Increased risk for thrombosis in some ITP patients
  - Increased prevalence of hyperthyroidism in ITP pts
    - 8-14% of pts with ITP develop thyroid dysfunction

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ITP & Helicobacter Pylori

- HP testing is more contentious
- A positive test is common with increasing age
  - screen test: HP serology
  - the C14 urea breath test to confirm active infections

- HP eradication has been associated with a platelet count rise in nearly 50% of HP positive pts (in the McMster ITP registry HP ITP 7/362)

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Laboratory diagnosis

- Surrogate tests for increased platelet turn over
  - Reticulated platelet
    - In ITP pts range 2.5 to 24.0%
    - In other thrombocytopenic pts range 1-9%

- Due to the wide rangers of these results, they have limited usefulness

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Laboratory test for ITP: autoantibodies

- No more used
  - Serological tests
  - Platelet associated immunoglobulins (PaIg)

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Laboratory test for ITP: autoantibodies

- Tests detecting Ig bound to platelet glycoproteins may be used
  - sensitivity < 50%,
  - specificity > 90%
    - MAIPA (Monoclonal Antibody-specific Immobilization of Platelet Antigen) and ELISA are the most common methods to detect platelet GpIIb/IIIa or GpIb/IX specific utoantibodies
    - Direct assyas have a better sensitivity than indirect

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Laboratory test for ITP: autoantibodies

- These tests are useful for ruling in but not for ruling out ITP.

- The interpretation for the low sensitivity is that in some pts:
  - antibodies are udetectable (because of low titer or sequestration)
  - antibodies are against other non-platelet target antigens (TPO or its receptor c-MPL)
  - Other pathological immune mechanisms independent of antibodies (cytotoxic T cells)

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Therapies for the treatment of ITP

Clinical situation	Therapy option
First line (initial treatment for newly diagnosed ITP)	Anti-D Corticosteroids: dexamethasone, methylprednisolone, prednis(ol)one IVIg
Second line	Azathioprine Cyclosporin A Cyclophosphamide Danazol Dapsone Mycophenolate mofetil Rituximab Splenectomy TPO receptor agonists Vinca alkaloids
Treatment for patients failing first- and second-line therapies	**Category A: treatment options with sufficient data** TPO receptor agonists **Category B: treatment options with minimal data and considered to have potential for considerable toxicity** Campath-1H Combination of first- and second-line therapies Combination chemotherapy HSCT

Blood 2010;115:168

Analoghi della TPO

- Romiplostin, Eltrombopag e AKR-501.
  - Romiplostin (Nplate) è un peptide che viene somministrato per via sottocutanea settimanalmente e che agisce legandosi ad un sito endogeno di legame della TPO.
  - Eltrombopag (Revolade) e AKR-501 sono due molecole non peptidiche a somministrazione orale giornaliera che agiscono legandosi rispettivamente ad una porzione intramembranaria e ad un sito endogeno del recettore della TPO.

- Questi farmaci sono potenzialmente utili non solo nella PPI ma anche nelle piastrinopenie associate a patologie epatiche ed al virus HCV, alla piastrinopenia in corso di chemioterapia o di anomalie intrinseche midollari.

- Sono poi allo studio anche anticorpi monoclonali che stimolino la megacariocitopoiesi legandosi direttamente al recettore per la TPO.

Romiplostim e eltrombopag

	Eltrombopag (formerly SB497115)[36,37,57-59]	Romiplostim (formerly AMG-531)[36,38,39,56,60-62]
Structure of the drug	Hydrazone organic compound, small molecule non-peptide agonist; interacts with c-MPL at a site different from thrombopoietin	Peptibody (recombinant protein); active peptides bind competitively with thrombopoietin to c-MPL; no sequence homology with thrombopoeitin; Fc fragments added to increase half-life
Molecular weight	564·6 Da	29 542 Da (monomer)
Effects on megakaryocyte	Stimulates megakaryoctye proliferation and differentiation	Increases megakaryoctye ploidy and maturation
Signalling pathway	Stimulates STAT5, mitogen-activated protein kinase p38	Induced phosphorylation of JAK2, STAT5
Route of administration	Once daily oral administration	Once per week subcutaneous injection
Dose	Optimum dose 30–75 mg	Median dose 2–3 μg/kg
Efficacy	Repeated administration increases platelet count starting on day 7, peak at day 16	Dose-dependent rise in platelet count starting at day 5 peak at day 12–15
After treatment discontinued	Platelet count returns to baseline within 2 weeks	Platelet count returns to baseline within 2 weeks
Development of antibodies against thrombopoietin	No	One report of transient neutralising antibody without affecting platelet count
Elimination	Free drug metabolised in liver, platelet-bound drug removed by the reticuloendothelial system	Unknown for free drug; platelet-bound drug removed by the reticuloendothelial system
Side-effects resulting from modifications of the megakaryocyte-platelet lineage	Possible increase in reticulin fibres in marrow*	Increased reticulin fibres in marrow of eight patients
Thrombosis	Rare patients recognised with added thromboembolic risk	12 patients had a thrombotic or thomboembolic event

cMPL=thrombopoietin receptor. *Published data not available for eltrombopag.

Table 2: Characteristics of eltrombopag and romiplostim

Lancet 2009;373:1562

	Mechanisms
First-line therapy for ITP	
Corticosteroids (eg, prednisone, prednisolone, dexamethasone, methylprednisolone)	Suppression of cell-mediated and humoral immunity, downregulation of Fc-mediated platelet clearance, anti-inflammatory action[1,2,5]
Treatment failure and severe thrombocytopenia	
Intravenous immunoglobulins (IVIgG)	Direct blockade of Fc-receptor mediated platelet clearance, presence of anti-idiotype antibodies, inhibition of T-cells and dendritic cells[20–23]
Anti-D (Rh)	Competitive interference in platelet destruction by the reticuloendothelial system[5,20,21]
Splenectomy	Removal of a major site of platelet sequestration and of antibody production[24–26]
Second-line options for refractory patients	
Use (or re-use) of first-line or other drugs alone or in combination (including azathioprine, cyclophosphamide, danazol, dapsone, vinca alkaloids)	Suppression of antibody production, decreasing Fc receptor densities, blocking macrophage function, chemotherapy[5,20,26]
Removal of accessory splenic tissue	Removal of a site of platelet sequestration[24–26]
Other more rarely used approaches	
Cyclosporin A, mycophenolate, interferon γ	Blockade of T-cell mediated responses, immunosuppression[24,27,28]
Humanised antibodies	
Rituximab* (anti-CD20 monoclonal antibodies)	B-cell depletion, inhibition of cellular immunity[5,20,29–32]
Anti-CD40 ligand (humanised monoclonal antibodies)	Blocking T-cell and platelet based stimulation of autoreactive B-cells[24,33–35]
New approaches: stimulation of megakaryocytopoiesis	
Romiplostim (AMG-531), eltrombopag (SB-497115)	c-MPL agonists, stimulation of megakaryocytopoiesis and platelet production[36–38]

For patients with severe bleeding, platelet transfusions, combinations of high doses of corticosteroids or IVIgG, or both, are initial treatment choices. Intravenous recombinant human factor VIIa can be considered.[3] cMPL=thrombopoietin receptor. *Rituximab is being increasingly used to treat refractory patients before splenectomy.[40] Order of citations does not indicate efficacy.

Table 1: Strategies to increase platelet counts in refractory idiopathic thrombocytopenic purpura (ITP)

Lancet 2009;373:1562

Table 4. First-line treatment options for adult ITP patients

Recommended treatment strategy	Approximate response rate	Approximate time to response	Toxicities	Duration of sustained response
Corticosteroids				
Dexamethasone 40 mg daily for 4 d every 2-4 wk for 1-4 cycles	Up to 90% of patients respond initially	Several days to several weeks	Vary with length of administration: mood swings, weight gain, anger, anxiety, insomnia, Cushingoid faces, dorsal fat, diabetes, fluid retention, osteoporosis, skin changes including thinning, alopecia, hypertension, GI distress and ulcers, avascular necrosis, immunosuppression, psychosis, cataracts, opportunistic infections, adrenal insufficiency; hypertension, anxiety. Tolerability decreases with repeated dosing. Possibly lower rate of adverse events when used as short-term bolus therapy	As high as 50%-80% reported, the latter with 3-6 cycles (during 2-5 y of follow-up)
Methylprednisolone 30 mg/kg/d for 7 d	As high as 95%	4.7 d vs 8.4 d (high-dose methylprednisolone [HDMP] vs prednisone)		23% of patients have sustained platelet count ($> 50 \times 10^9$/L) at 39 mo
Prednis(ol)one 0.5-2 mg/kg/d for 2-4 wk	70%-80% of patients respond initially	Several days to several weeks		Remains uncertain; estimated 10-y disease-free survival 13%-15%
IV anti-D				
50-75 μg/kg	Initial response rate similar to IVIg (dose dependent)	4-5 d	Common: hemolytic anemia (dose-limiting toxicity), fever/chills Rare: intravascular hemolysis, disseminated intravascular coagulation, renal failure, rare death	Typically last 3-4 wk but may persist for months in some patients
IVIg*				
0.4 g/kg/d for 5 d or infusions of 1 g/kg/d for 1-2 d	Up to 80% of patients respond initially; half achieve normal platelet counts	Rapid; many respond in 24 h; typically 2-4 d	Headaches common: often moderate but sometimes severe Transient neutropenia, renal insufficiency, aseptic meningitis, thrombosis, flushing, fever, chills, fatigue, nausea, diarrhea, blood pressure changes and tachycardia IVIg preparations may contain small quantities of IgA, which occasionally causes anaphylactoid reactions in patients with IgA deficiency; in these cases use IgA-depleted IVIg	Usually transient; platelet counts returning to pretreatment levels 2-4 wk after treatment; persists for months in a few patients

Full details regarding the studies found in the literature search are available in supplemental Document 3.
*IVIg may be discontinued after 1 to 2 days if adequate response is seen.

Provan D, et al. Blood.2010;115(2):168-186.

Table 1. American Society of Hematology Guidelines for the Management of Newly Diagnosed ITP in Adults and Children (adapted from the American Society of Hematology Guidelines for Immune Thrombocytopenia[5])

Children

We recommend:

- Children with no bleeding or mild bleeding (defined as skin manifestations only, such as bruising and petechiae) be managed with observation alone regardless of platelet count (grade 1B);
- In pediatric patients requiring treatment, a single dose of IVIg (0.8-1.0) or a short course of steroids be used as first-line treatment (grade 1B);
- IVIg can be used if a more rapid increase in the platelet count is required (grade 1B);
- Anti-D immunoglobulin therapy is not advised in children with a hemoglobin concentration that is decreased due to bleeding or with evidence of autoimmune hemolysis (grade 1C).

We suggest:

- A single dose of anti-D immunoglobulin can be used as first-line treatment in Rh-positive, nonsplectomized children requiring treatment (grade 2B).

Adults

We suggest:

- Treatment be administered to for newly diagnosed patients with a platelet count $<30 \times 10^9$/l (grade 2C);
- Longer courses of steroids are preferred over shorter courses of corticosteroids or IVIg as first-line treatment (grade 2B);
- IVIg can be used with corticosteroids when a more rapid increase in the platelet count is required (grade 2B);
- Either IVIg or anti-D immunoglobulin (in appropriate patients) be used as first-line treatment if corticosteroids are contraindicated (grade 2C);
- If IVIg is used, the dose should be initially 1 gm/kg as a 1-time dose; this dosage may be repeated if necessary (grade 2B).

Neunert C, et al. Blood. 2011;117(16):4190-4207.

Table 2. Splenectomy vs TPO-RA and rituximab in refractory/relapsed ITP

Therapy	Response rate and durability	Time to response	Adverse effects	Contraindications	Preferred in	Approximate cost
Splenectomy	Overall response rate >80%, 50%-75% at 5 y	Days	Surgical mortality (<0.2% with laparoscopic splenectomy), surgery-related complications (9.6%; bleeding, infection, thrombosis) Lifetime risk of overwhelming sepsis Possible vascular complications: VTE, ATE	Multiple comorbidities, poor surgical candidate Relative: advanced age (higher rate of complications, lower response rate at age >60-70) *Helicobacter pylori*, hepatitis C (treat underlying cause first)	Fulminant ITP refractory to corticosteroids/IVIg with poor response to TPO-RA, desire to avoid drug therapy or close medical monitoring, uncertain compliance with medical therapy, prohibitive cost of medical therapy	20 000 USD
TPO-RA (eltrombopag and romiplostim)	80% overall response rate, high rates of durable response on continued therapy	10-14 d	Headache, rebound thrombocytopenia, elevated liver enzymes (eltrombopag), bone marrow reticulin fibrosis, possible small increased risk of venous thrombosis	Pregnancy (category C) and lactation, MDS Caution in patients with liver disease and a history of thrombosis	Patient preference, patients not interested in or unable to undergo splenectomy	Annually ~108 000 USD*
Rituximab	60% overall response rate; 21%-26% of responders at 1 y have responses at 5 y	1-8 wk	Infusion-related adverse events (fever, chills, dyspnea, hypotension), neutropenia, hypogammaglobulinemia, reactivation of viral infections (hepatitis B), progressive multifocal leucoencephalopathy (rare)	Active hepatitis B infection, pregnancy (category C) and lactation	Patient preference, patients not interested in or unable to undergo splenectomy, patient seeks medical long-term remission	10 000-40 000 USD per 4-infusion course

MDS, myelodysplastic syndrome; USD, United States dollars.

*Cost is estimated based on average wholesale cost for the following doses: eltrombopag 50 mg daily and romiplostim 3 μg/kg per week for a 70-kg individual.

Chaturvedi S et al. Blood. 2018;131(11):1172-1182)

Definitions of time to and duration of response, and the time to initial and peak response for different ITP treatments

Time to response	From start of treatment until either complete response or response		
Duration of response	Time from complete response or response until loss of complete response or response Measured as the proportion of the cumulative time spent in complete response or response during the period under examination as well as the total time observed from which the proportion is derived		
Expected time to response	**Treatment type**	**Initial response, days**	**Peak response, days**
	Anti-D	1-3	3-7
	Azathioprine	30-90	30-180
	Danazol	14-90	28-180
	Dexamethasone	2-14	4-28
	Eltrombopag	7-28	14-90
	IVIg	1-3	2-7
	Prednisone	4-14	7-28
	Rituximab	7-56	14-180
	Romiplostim	5-14	14-60
	Splenectomy	1-56	7-56
	Vinblastine	7-14	7-42
	Vincristine	7-14	7-42

Blood. 2011;117(16):4190-4207

Definitions of response to treatment

Complete response (CR)	A platelet count $\geq 100 \times 10^9$/L measured on 2 occasions $>$ 7 days apart and the absence of bleeding.
Response (R)	A platelet count $\geq 30 \times 10^9$/L and a greater than 2-fold increase in platelet count from baseline measured on 2 occasions $>$ 7 days apart and the absence of bleeding.
No response (NR)	A platelet count $< 30 \times 10^9$/L or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart.
Loss of complete response	A platelet count $< 100 \times 10^9$/L measured on 2 occasions more than a day apart and/or the presence of bleeding.
Loss of response	A platelet count $< 30 \times 10^9$/L or a less than 2-fold increase in platelet count from baseline or the presence of bleeding. Platelet count must be measured on 2 occasions more than a day apart.

Blood. 2011;117(16):4190-4207

Piastrinopenie da farmaci su base immunologica

- insorgono in seguito a somministrazione di farmaci capaci di indurre, con meccanismo immunologico, una piastrinopenia da consumo periferico.

- È una patologia piastrinica di non rara osservazione.
- Numerosi farmaci sono stati implicati come causa di piastrinopenia su base immunologica.
  - Generalmente il paziente sensibilizzato da una precedente assunzione del farmaco, manifesta una caduta improvvisa del tasso di piastrine con sintomatologia emorragica, in occasione della riassunzione del farmaco stesso.
  - L'intensità dei diversi quadri clinici e la durata della fase piastrinopenica è molto variabile da paziente a paziente.
  - Vengono distinti diversi tipi di piastrinopenia da farmaci mediata da anticorpi.

Principali farmaci indicati come possibili cause di piastrinopenia

Penicilline
- Penicillina sodica
- Ampicillina
- Carbenicillina
- Meticillina

Cefalosporine
- Cefalexina
- Cefalotina
- Cefamandolo
- Moxalactam

Aminoglicosidi
- Streptomicina
- Gentamicina

Macrolidi
- Rifampicina

Glicopeptidi
- Vancomicina

Sulfamidici
- Trimetoprim
- Sulfasalazina

Antiprotozoari
- Chinina
- Clorochina
- Idrossiclorochina

Analgesici e FANS
- Indometacina
- Acido acetilsalicilico
- Noraminopirina
- Acetaminofene
- Fenilbutazone
- Piroxicam
- Ibuprofene
- Diclofenac

Sedativi e tranquillanti
- Allilisopropilacetilurea
- Allilisopropilbarbiturato
- Carbamazepina
- Clorpromazina
- Diazepam
- Difenilidantoina
- Acido valproico

Antidiabetici
- Clorpropamide
- Glibenclamide
- Tolbutamide

Antagonisti di GpIIa/IIIb
- Abiciximab
- Eptifibatide
- Tirofiban

Cardiovascolari
- Chinidina
- Digitale
- Digitossina e digossina
- a-metildopa
- Diltiazem
- Captopril
- Oxprenololo
- Amiodarone
- Procainamide

Diuretici
- Furosemide
- Clortalidone
- Clorotiazide
- Idroclorotiazide
- Spironolattone

Altri
- Cimetidina e ranitidina
- Sali d'oro
- Eparina
- Ticlopidina
- Eroina

meccanismi implicati nella piastrinopenia da farmaci immunologica

tipo	meccanismo	farmaco	Incidenza
Anticorpo aptene dipendente	L'aptene si lega in modo covalente alla proteina di membrana ed induce una risposta immunologica farmaco specifica	Penicilline cefalosporine	Molto rara
Farmaci tipo chinino	Il farmaco induce la produzione di anticorpi che si legano alla proteina di membrana in presenza del farmaco in forma solubile	Chinino sulfonamidici FANS	26 casi per milione di pazienti che assumono chinino
Farmaci tipo fiban	Il farmaco reagisce con la GpIIb/IIIa ed induce una modificazione conformazionale (neoepitopo) riconosciuta dall'anticorpo	Tirofifan, eptifibatide	0.2-0.5%
Anticorpi farmaco specifici	L'anticorpo riconosce componenti di origine murina del frammento chimerico Fab specifico per la GpIIIa	Abciximab	0.5-1.0% dopo la prima esposizione, 10-14% dopo la seconda esposizione
Autoanticorpi	Il farmaco induce la formazione di anticorpi che reagiscono con le piastrine autologhe in assenza del farmaco	Sali d'oro Procainamide	1.0 % con i Sali d'oro, rara con la procainamide o altri farmaci
immuncomplessi	Il farmaco si lega al fattore piastrinico 4 producendo immuncomplessi per i quali l'anticorpo è specifico; l'immuncomplesso attiva le piastrine attraverso i recettori Fc	Eparina	3-6% tra i pazienti trattati con eparina non frazionata per 7 gg rara con le eparine a basso peso molecolare

Piastrinopenia da farmaci

- Diagnosi, decorso e prognosi
  - Un’accurata anamnesi farmacologica per la diagnosi differenziale dalle altre piastrinopenie.
  - La prognosi è in genere favorevole, per la rapida regressione della piastrinopenia e della sintomatologia correlata dopo sospensione del farmaco.

- Terapia
  - In generale non è indicata alcuna terapia particolare, oltre l’ovvia sospensione della somministrazione del farmaco.

Piastrinopenia da eparina

- La piastrinopenia da eparina (heparin-induced thrombocytopenia, HIT) è una delle più comuni e potenzialmente devastanti reazioni a farmaci di tipo immunomediato.

- A differenza delle altre piastrinopenie da farmaci immunomediate, la HIT non causa emorragie ma trombosi.

Piastrinopenia da eparina tipo I

- è la forma più comune,
- caratterizzata da
  - piastrinopenia moderata (raramente il tasso di piastrine scende sotto 100 x 10^9/l),
  - esordio precoce
  - reversibilità del fenomeno senza necessità di sospendere il trattamento;

Patogenesi

- Il meccanismo eziopatogenetico della HIT di tipo I è sconosciuto

- si ammette che sia di tipo non immune ed ascrivibile al ruolo proaggregante piastrinico esercitato dall'eparina in pazienti nei quali vi sia stata una precedente attivazione piastrinica con un conseguente aumentato sequestro a livello splenico.

Piastrinopenia da eparina tipo II (HIT)

- meno frequente,
- ha un'insorgenza più tardiva, a 5-10 gg dall'inizio della terapia
- non recede anche dopo sospensione del farmaco
- è più grave per il grado di piastrinopenia (<100 x 10^9/l)
- quadro clinico contrassegnato da
  - trombosi arteriose periferiche che possono portare a gangrena,
  - ischemia miocardica o cerebrale,
  - più frequentemente da trombosi venose profonde, localizzate in varie sedi, spesso complicate da embolia polmonare.
  - La mortalità è elevata (sino al 30%).
- I pazienti a rischio maggiore sono quelli che hanno subito interventi chirurgici, soprattutto ortopedici, o con infezioni.

Patogenesi HIT

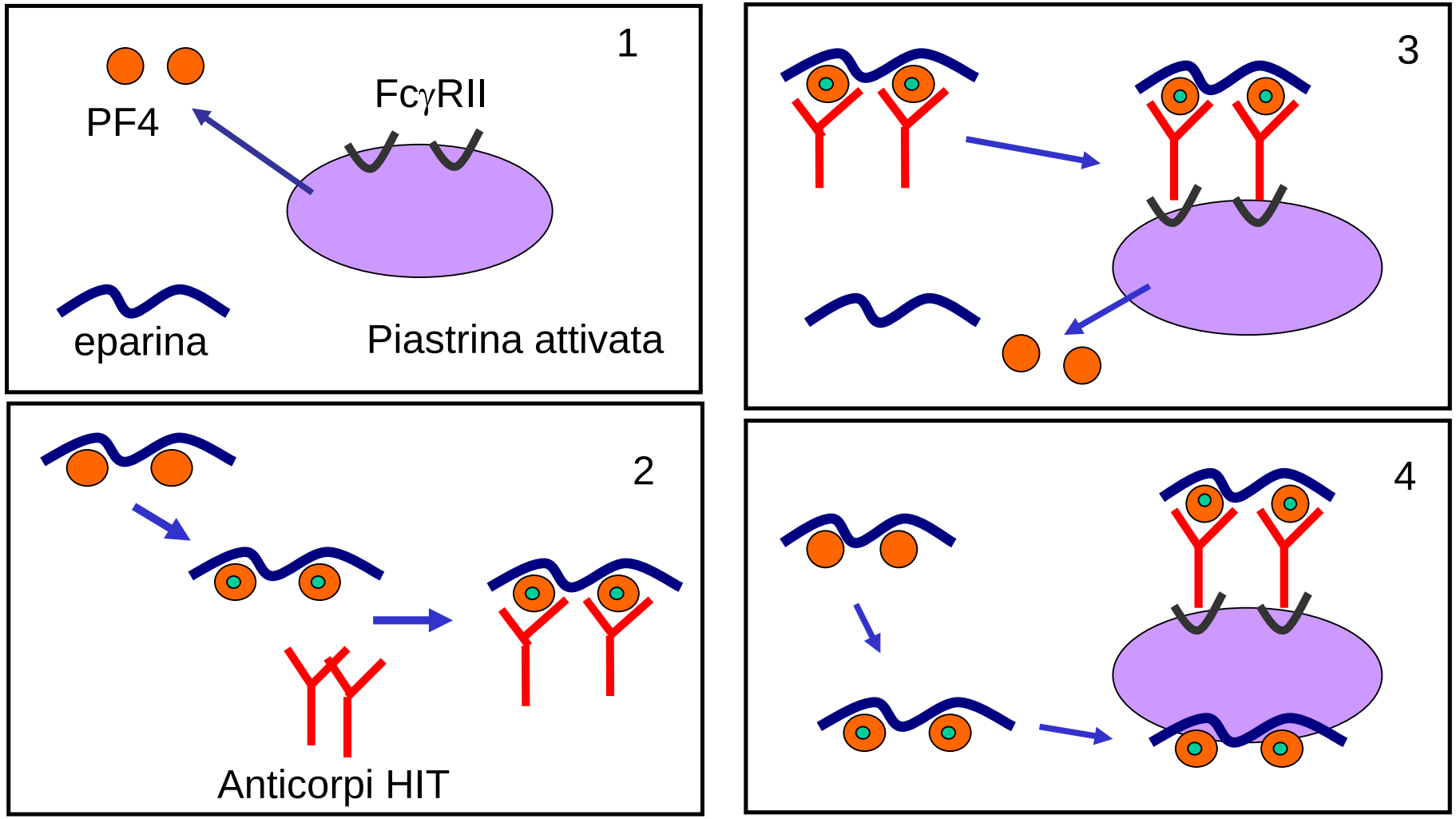
- è legata al
  - legame tra l'eparina ed il fattore piastrinico 4 (PF4), rilasciato dai granuli α a seguito dell'attivazione piastrinica,
  - alla conseguente formazione di un complesso PF4-eparina
  - che induce, nel PF4, un cambiamento conformazionale
  - che porta all'esposizione di epitopi criptici
  - che determinano una risposta autoanticorpale.

Patogenesi HIT

- Si genera quindi una catena di eventi che porta tra l'altro anche alla produzione di molecole ad azione procoagulante con conseguente generazione di trombina.

- Questo processo protrombotico sarebbe alla base dello stato di ipercoagulabilità e delle complicanze trombotiche presenti nella HIT.

Piastrinopenia da eparina tipo II: eziopatogenesi

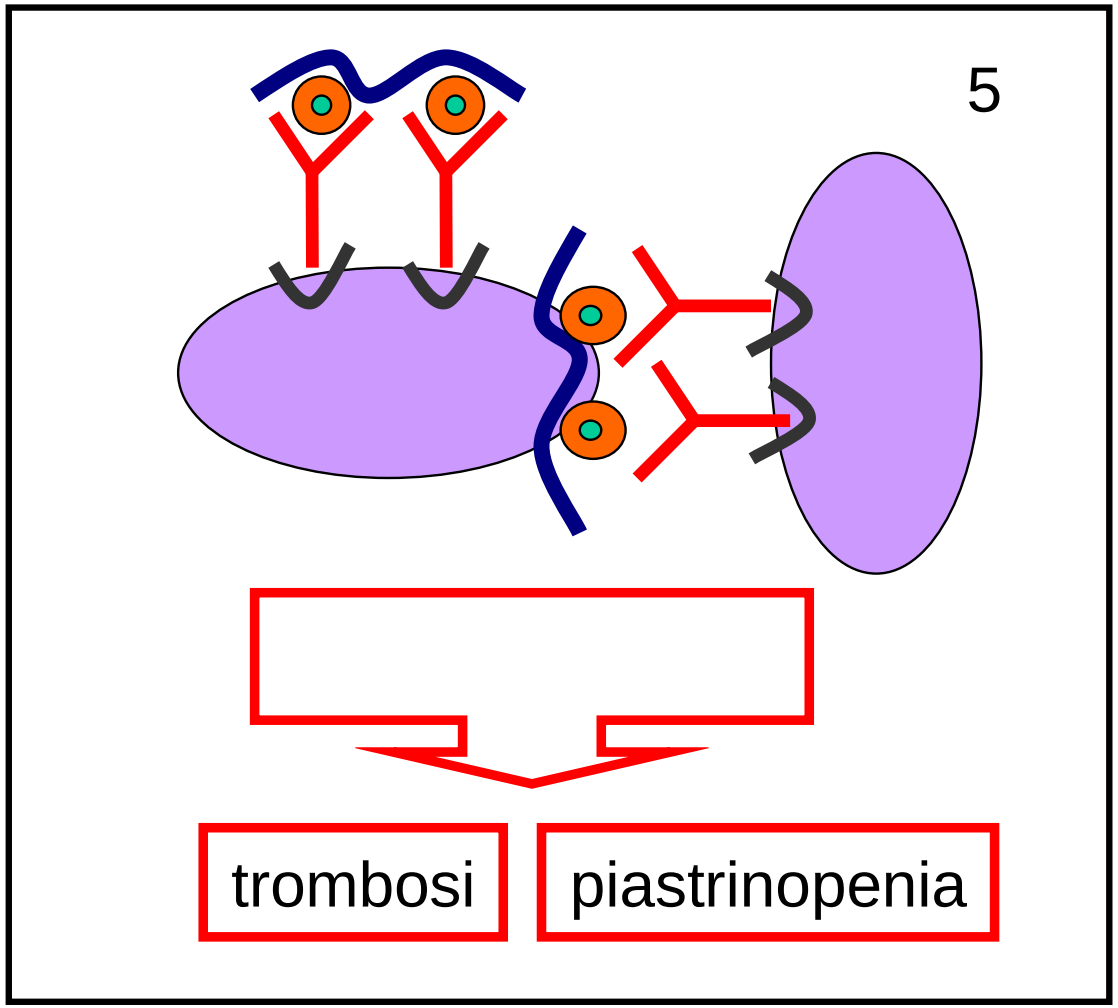


Patogenesi HIT

- La trombocitopenia è invece dovuta alla clearance, da parte del sistema reticolo endoteliale, delle piastrine attivate e delle piastrine cui sono legati gli anticorpi.

- Le eparine a basso peso molecolare (LMWH) presentando una lunghezza inferiore rispetto alle eparine non frazionate si legano debolmente al PF4, e avendo una minore antigenicità causano meno frequentemente HIT.

Piastrinopenia da eparina: eziopatogenesi



Patogenesi HIT

- La maggior parte dei pazienti (>80%) presenta anticorpi di classe IgG.
- In alcuni pazienti (<20%) sono presenti solo anticorpi IgA e IgM.
- Gli anticorpi delle classi IgA e IgM non determinano attivazione piastrinica in quanto le piastrine non hanno recettori Fc per queste classi di immunoglobuline.
- Questo renderebbe ragione del fatto che i pazienti con anticorpi IgA e IgM sono generalmente asintomatici.
- Gli anticorpi anti complesso eparina-PF4 possono rimanere in circolo per 3-4 mesi anche dopo la discontinuazione del trattamento eparinico.

Diagnosi

- La diagnosi di HIT deve essere presa in considerazione nel caso in cui un paziente, che stia ricevendo eparina da 5 almeno giorni,
  - manifesti un calo piastrinico al di sotto delle 150 x 10^9/l (decremento piastrinico minimo di 30 x 10^9/l)
  - O nel caso in cui si abbia un decremento piastrinico superiore al 50% dei valori basali.

- La piastrinopenia può talora comparire prima di 5 giorni se il paziente ha ricevuto eparina nei precedenti 120 gg e se presenta anticorpi circolanti.

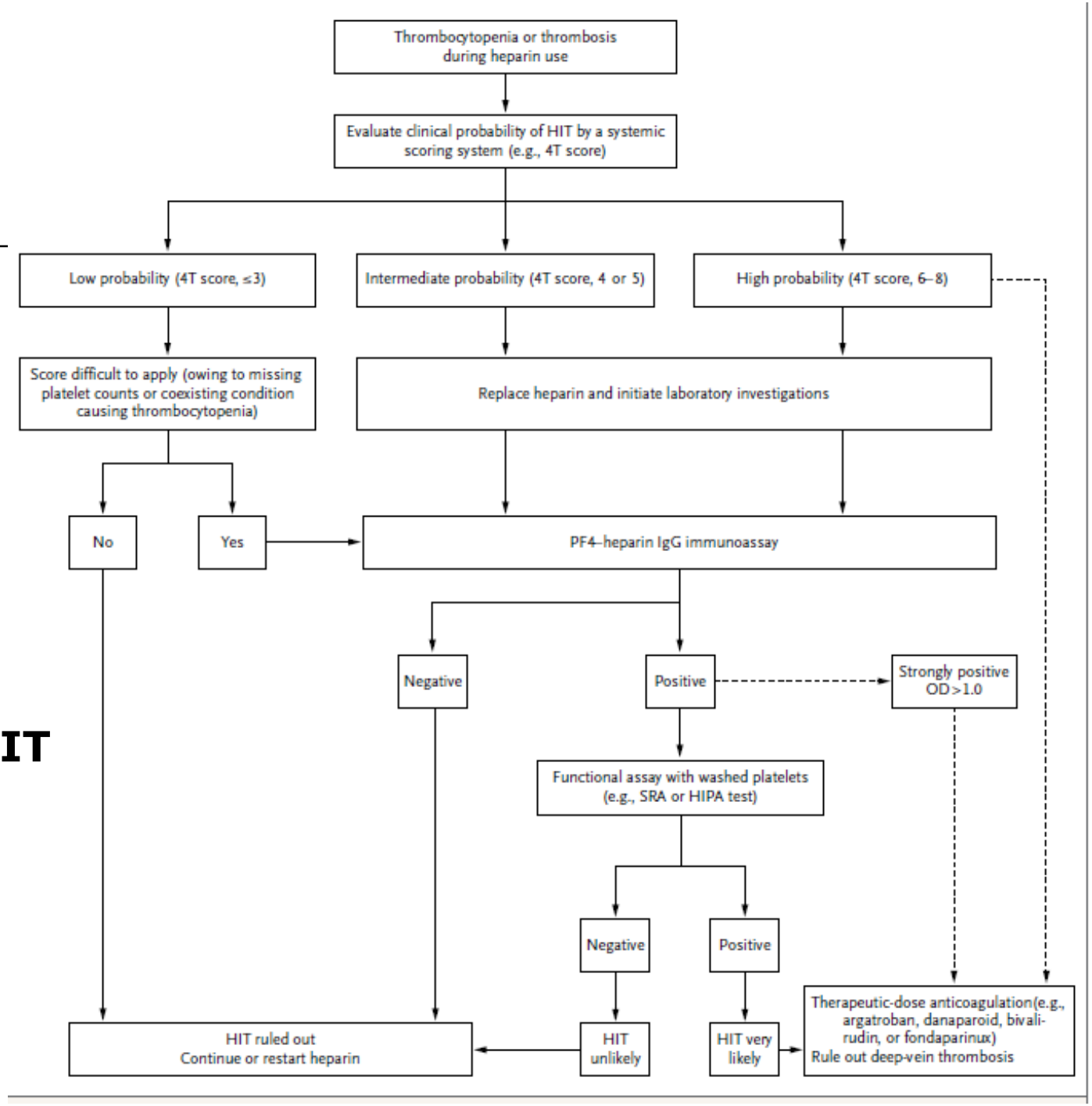
- È necessario escludere altre cause di piastrinopenia.

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

N Engl J Med 2015;373:252-61.

Diagnosis of HIT



N Engl J Med 2015;373:252-61.

Terapia

- La prescrizione immediata è quella di sospendere il trattamento con eparina e di applicare un trattamento anticoagulante alternativo
- Quando il paziente è clinicamente stabile, e la trombosi e la piastrinopenia sotto controllo, si potrà passare ai coumadinici.
- Le LMWH non possono essere usate in pazienti con HIT a causa della cross-reattività degli anticorpi con il complesso LMWH-PF4.
- Le LMWH possono essere invece utilizzate, pur in presenza di una storia positiva per HIT, se il paziente non presenta anticorpi circolanti.

Anticoagulant management in HIT

- Several possible anticoagulant options exist for treatment of both HIT with thrombosis (HIT-T) or without thrombosis (isolated HIT).
  - **Argatroban** and danaparoid are approved for the treatment of HIT,
  - **Bivalirudin** is approved for treatment of HIT for patients undergoing percutaneous coronary intervention.
  - **Danaparoid** is not available in the United States.
  - **Fondaparinux** and desirudin are "off label" for the treatment of HIT but have data to support their use.

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Table 4. Parental anticoagulants for the management of HIT

	Indirect factor Xa inhibitors		Direct thrombin inhibitors		
	Fondaparinux	Danaparoid	Argatroban	Bivalirudin	Desirudin
Administration	Subcutaneous injection	IV infusion or subcutaneous injection	Continuous IV infusion	Continuous IV infusion	Subcutaneous injection
Clearance (half-life)	Renal (17 h)	Renal (24 h)	Hepatobiliary (40-50 min)	Renal/enzymatic (25 min)	Renal (2 h)
INR interference	No	No	Yes	Yes	Possible
Drug monitoring	None*	Anti-Xa activity*	PTT	PTT	None
Reversal agent	None†	None†	None	None	None
Other	Caution with renal impairment. Contraindicated with CrCl <30 mL/min	Caution with renal impairment; not available in the United States	Caution with liver dysfunction; consider dose reduction in critically ill patients	Evidence for use available in cardiac surgery and PCI	Caution with renal impairment

CrCl, creatinine clearance; PCI, percutaneous coronary intervention.

*Some centers monitor fondaparinux using fondaparinux-specific anti-Xa activity. Some centers do not routinely monitor danaparoid, particularly in patients with normal renal function.[93]

†Andexanet alfa is a reversal agent for direct and indirect Xa inhibitors but has not been studied or approved for use in HIT or with reversal of fondaparinux or danaparoid. Given the potential risk of ischemic events with andexanet alfa, caution in HIT is needed.

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Anticoagulant management in HIT: fondaparinux

- There is reasonably strong data to support the use of fondaparinux in the treatment of HIT.
  - Fondaparinux is an indirect factor Xa inhibitor that has proven efficacy in the treatment of other conditions such as VTE and acute coronary syndromes.
  - Fondaparinux is given by subcutaneous injection and does not affect the PTT and may provide more stable dosing in severe HIT where coagulation parameters can be abnormal at baseline.
  - The development of heparin antibodies that cross-react to fondaparinux is exceedingly rare (3 cases reported);
    - autoimmune HIT or an alternative diagnosis should be considered if the platelet count does not recover after fondaparinux is initiated.

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Anticoagulant management in HIT

- The DOACs are attractive for use in HIT because they have a quick onset of action, do not lower protein C levels, and are not known to cause antibodies.

- Some studies have investigated DOAC, however the limitation of these studies included a selected patient population where DOACs were deemed appropriate, and few HIT patients had an initial ATE.

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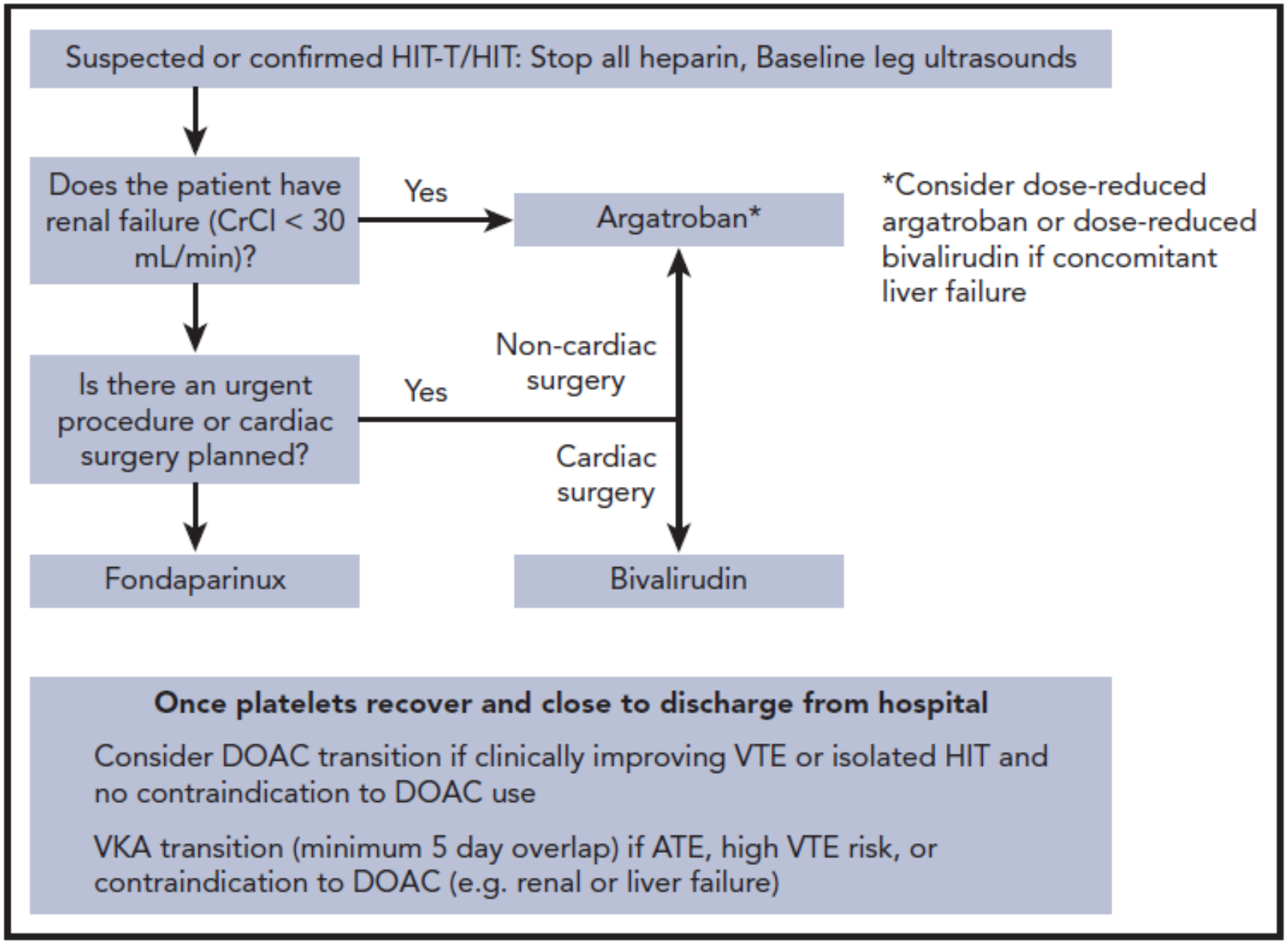

Figure 2. My management approach for suspected or confirmed HIT.

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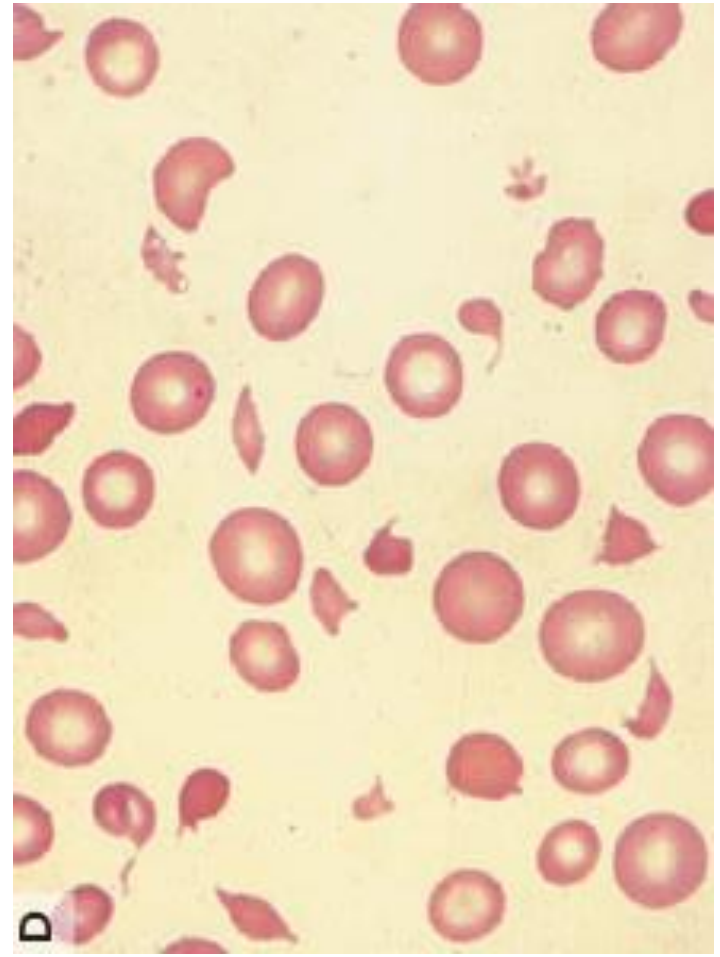
Microangiopatie trombotiche: definizione

- disordini occlusivi del microcircolo vascolare dovuti alla
  - Aggregazione piastrinica sistemica e/o intrarenale
- Con consegente
  - Piastrinopenia
  - Anemia da danno meccanico a carico degli eritrociti



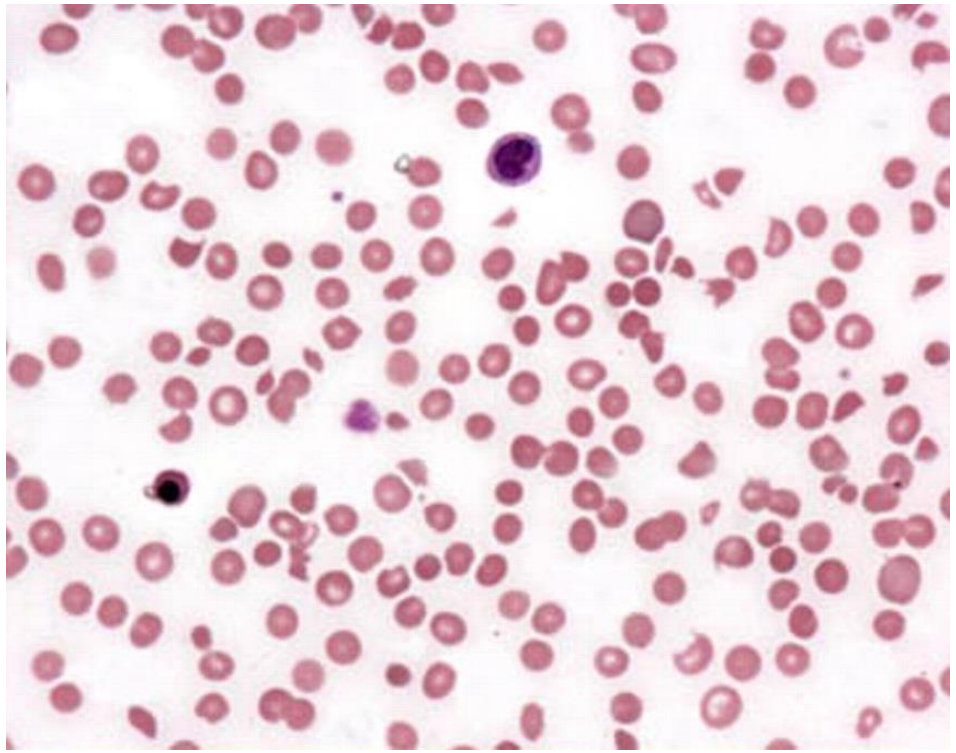
Condizioni cliniche associate a microangiopatia trombotica

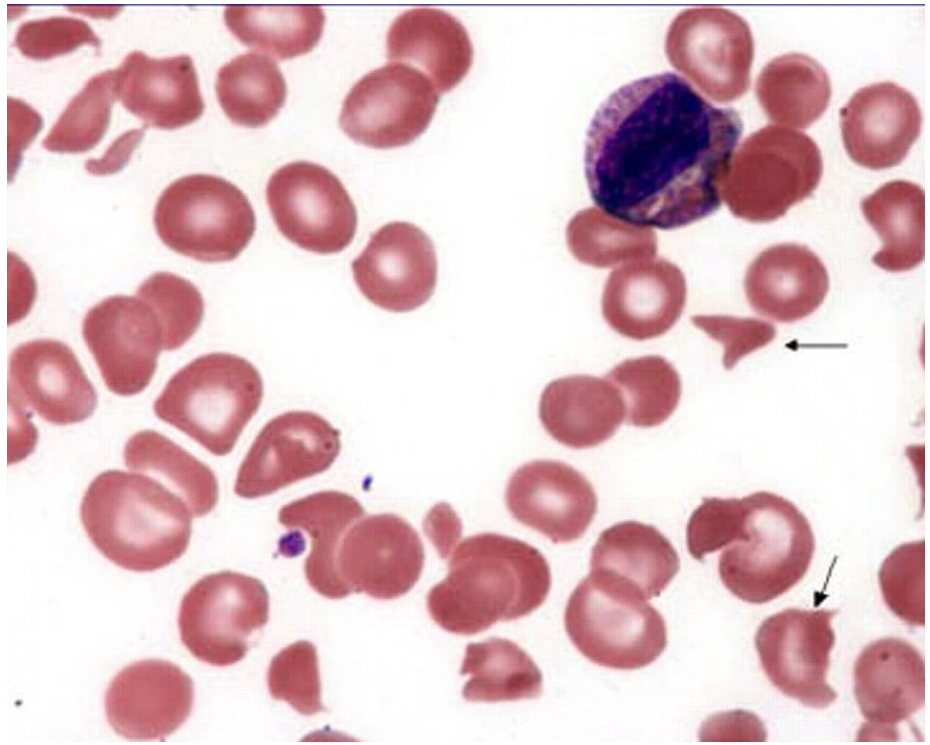
Condizione Clinica	Causa
Neoplasie	Metastasi
Farmaci e sostanze varie	Ciclosporina, tacrolimus, chemioterapici, statine, ticlopidina, clopidogrel, cocaina
Trapianto	Di cellule staminali allogeniche, di organi solidi
Interventi cardiovascolari recenti	Cateterizzazioni cardiache, angioplastica, bypass, valvole cardiache
CID	Consumo fibrinogeno
Disordini trombotici	Emoglobinuria parossistica notturna
Infezioni	Sepsi, AIDS
Collagenopatie	LES, artrite reumatoide, sclerodermia
Ipertensione severa	> 200/120 mmHg
Vasculiti	crioglobulinemia
Gravidanza, post-partum	Pre-eclampsia, HELLP syndrome
Sindrome da anticorpi antifosfolipi	
Carenza di Diacylglycerol kinase E (DGKE)	
Difetto di cobalamina C	
Sindrome emolitico uremica (HUS)	STEC-HUS, HUS secondarie, HUS atipica
Porpora trombotica trombocitopenica	Ereditaria od Acquisita

Presentazione clinica

- Il quadro clinico è caratterizzato da
  - Piastrinopenia
    - con incremento del numero di megacariociti a livello midollare
  - Anemia emolitica microangiopatica
    - Frammentazione degli eritrociti (schistociti, o cellule ad elmetto),
    - I globuli rossi frammentati si formano per il flusso turbolento nelle le zone del microcircolo parzialmente occluse per aggregazione piastrinica
  - Livelli elevati di lattico deidrogenasi serica.
    - principalmente da tessuto ischemico o necrotico piuttosto che dalla lisi cellulare
  - Sintomi neurologici
    - Ischemia cerebrale
  - Insufficienza renale
  - febbre
- La severità di queste anomalie riflette l'estensione della aggregazione microvascolare delle piastrine.

Peripheral smear showing RBC fragmentation consistent with a microangiopathic hemolytic process





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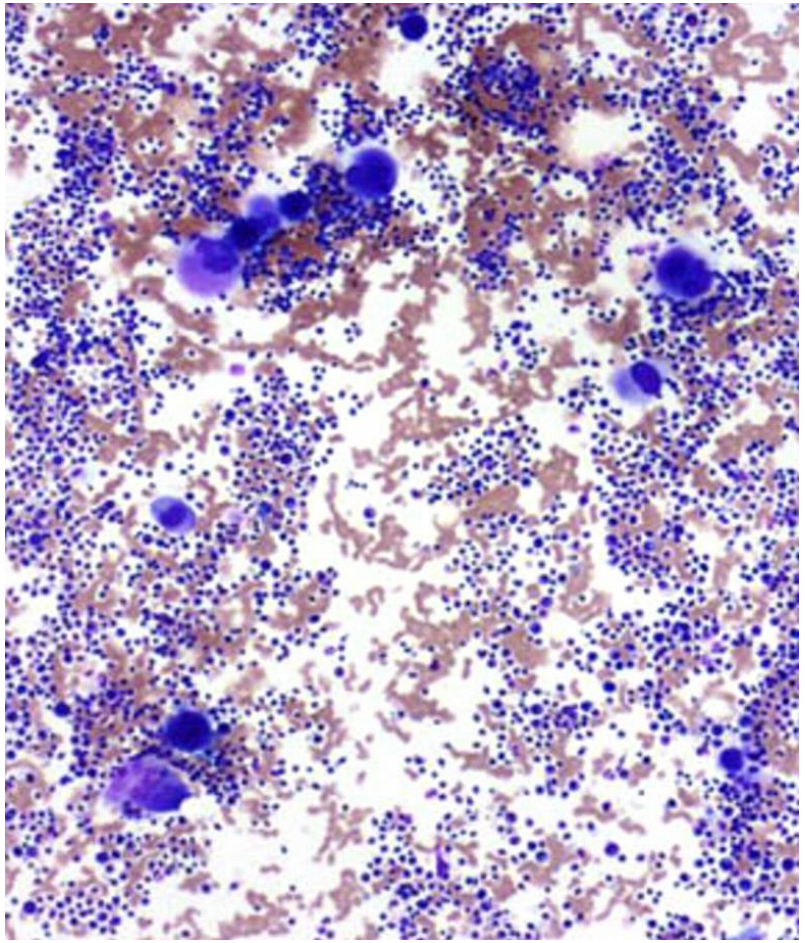
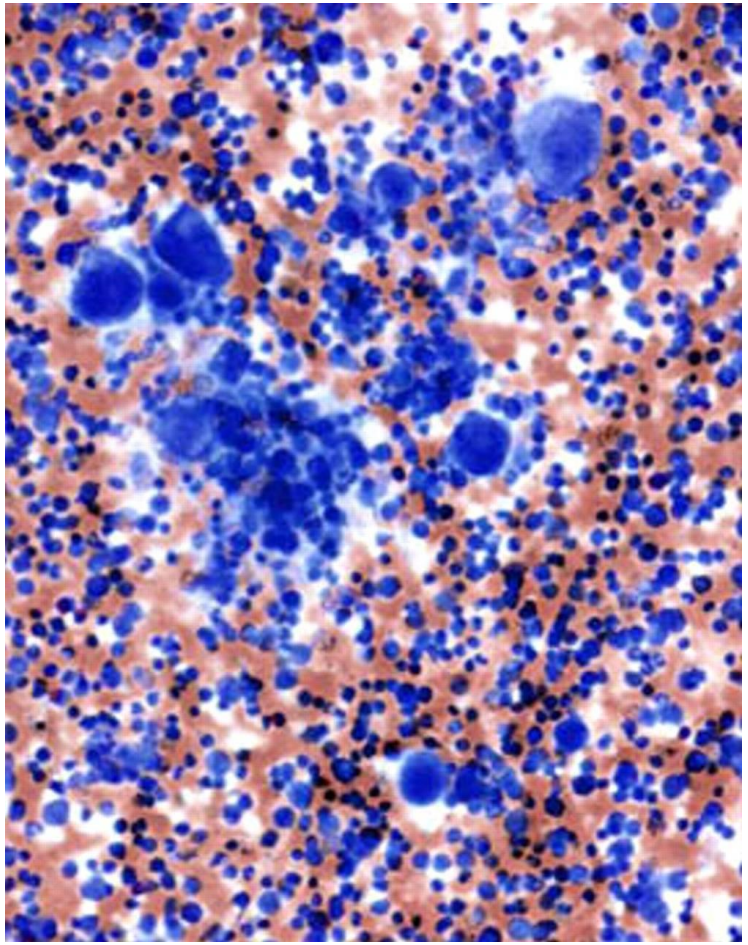
Bone marrow aspirate showing marked megakaryocytic hyperplasia





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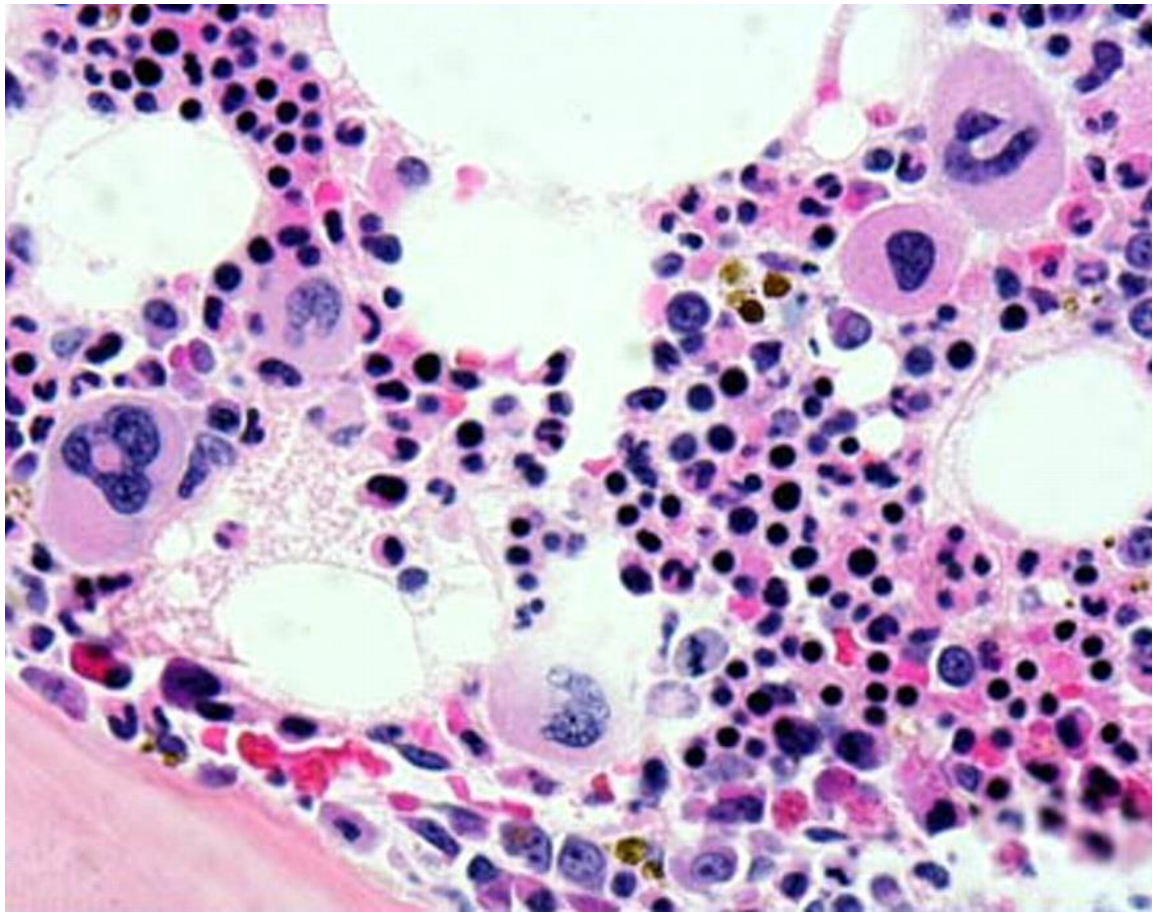
Bone marrow biopsy with megakaryocytic and erythroid hyperplasia



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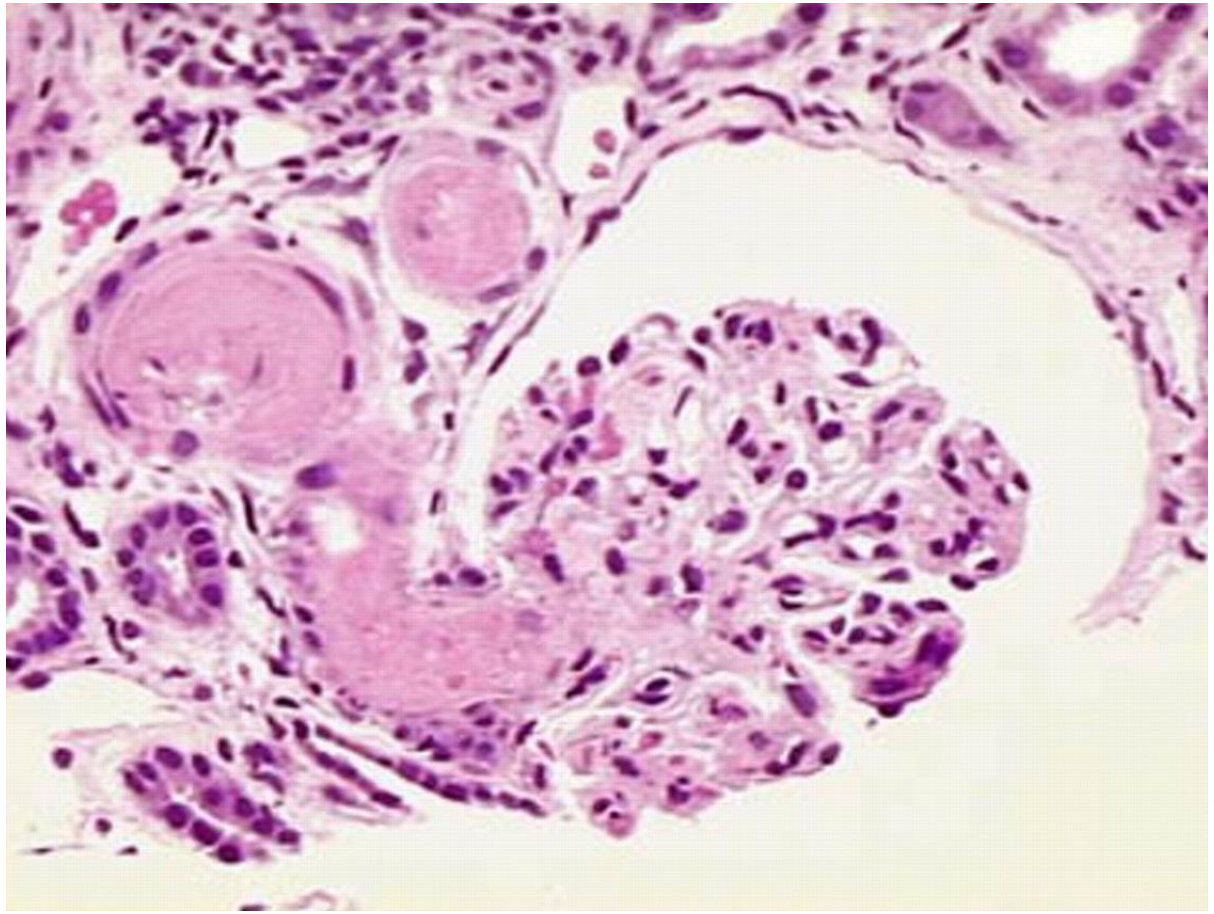
Renal biopsy showing hyaline thrombi in the glomerulus and small arterioles



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TTP: definition

- The definition for TTP has changed over time.
- Initially, an acute episode of TTP was defined by clinical criteria (multivisceral ischemic symptoms mainly targeting the brain) and standard biology criteria (microangiopathic hemolytic anemia and severe thrombocytopenia) occurring in the absence of other apparent causes.

- This definition was recently completed by the presence of a severe deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with a ThromboSpondin type 1 motif, member 13: activity <10%), which is the only biologic marker specific for TTP.

Joly et al. Blood. 2017;129(21):2836-2846

Acquired TTP epidemiology

- The incidence is approximately 3 cases per 1 million adults per year
- The median age is 41, with a wide range (9 to 78 yrs).
- Acquired TTP is very rare in children.
  - The incidence of acquired TTP in children <18 yrs is approximately 1 per 10 million per year (ie, 30-fold less common than in adults).
  - In children, the possibility of hereditary rather than acquired TTP must be considered.
- Demographic features associated with an increased risk of TTP include female sex and black race

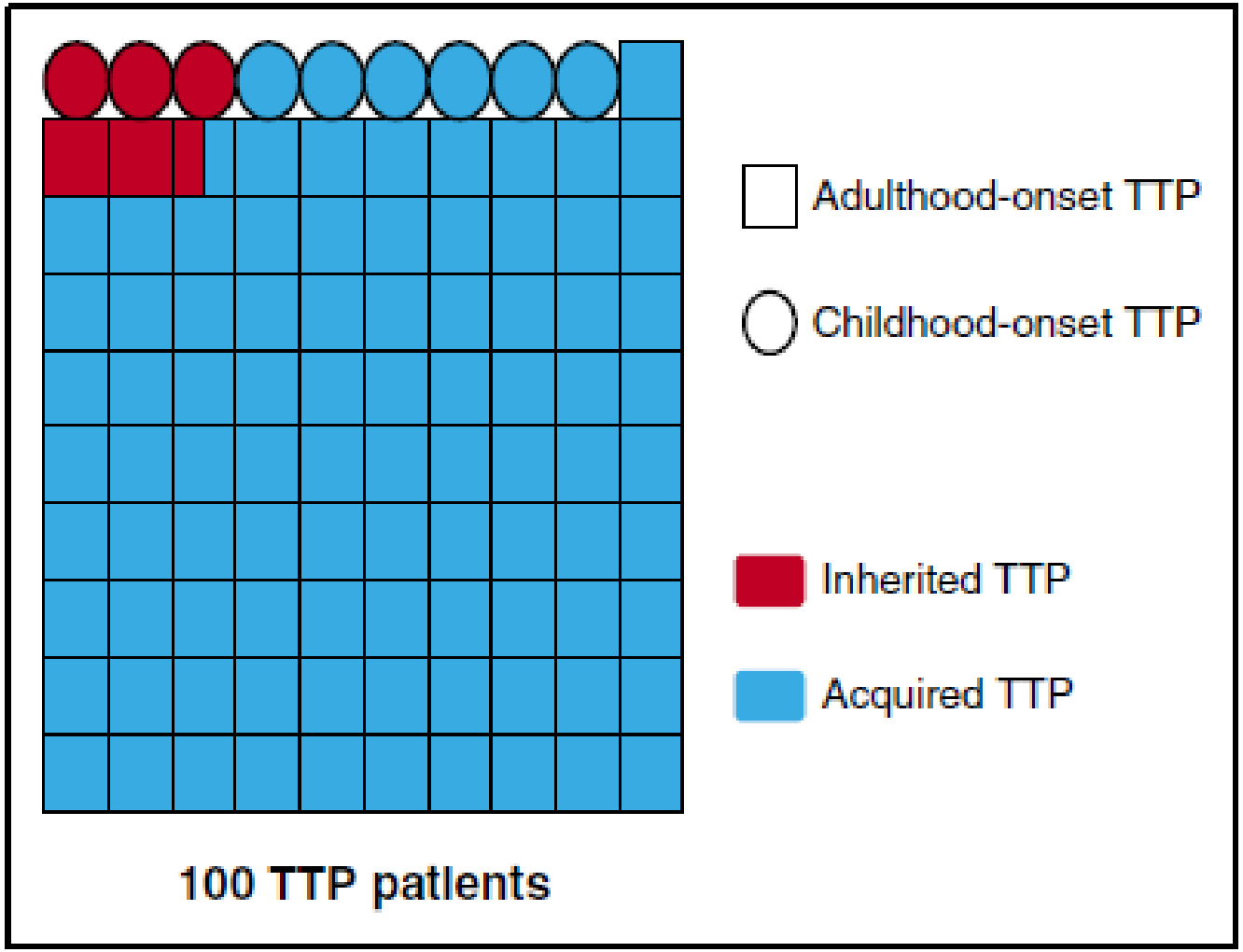
Hereditary TTP

- Pts with severe ADAMTS13 deficiency (typically, undetectable activity) due to inherited ADAMTS13 gene mutations.
- This condition is also called congenital TTP, inherited TTP, familial TTP, and Upshaw-Schulman syndrome (USS).
- Autosomal recessive condition.
  - Both alleles of the ADAMTS13 gene must be disrupted to cause deficiency severe enough to lead to the clinical syndrome. Either homozygous mutations or compound heterozygous mutations in ADAMTS13 may be responsible. In contrast, individuals who are heterozygous for an ADAMTS13 mutation appear to be unaffected by conditions that can precipitate TTP episodes in patients with biallelic ADAMTS13 defects.
- A variety of mutations in ADAMTS13 have been described, including insertions, deletions, missense and nonsense point mutations, and splice site mutations

Hereditary TTP: epidemiology

- Very rare disorder.
  - Only 150 families worldwide, although this may be an underestimate
- represents less than 5% of all TTP cases
- Among certain groups such as newborn infants and young children, hereditary TTP may be more common than acquired TTP.
- In pregnant women, hereditary TTP may represent up to 25% of TTP cases.
- There do not appear to be any ethnic, racial, or geographic differences in the prevalence of hereditary TTP.
- Some affected families may have a history of consanguinity.

TTP as a function of age of onset and mechanism for ADAMTS13 deficiency.



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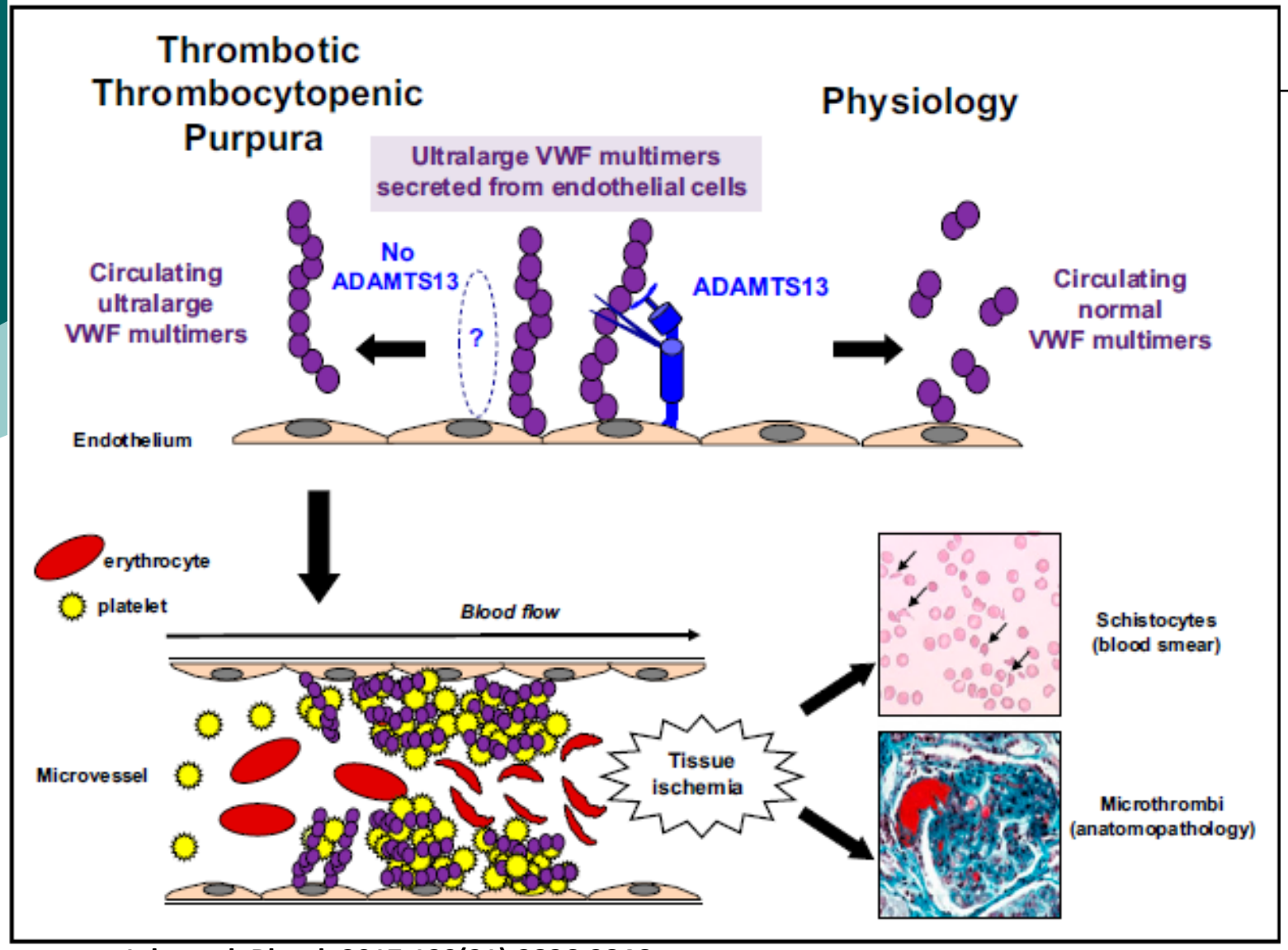
Pathophysiology for TTP



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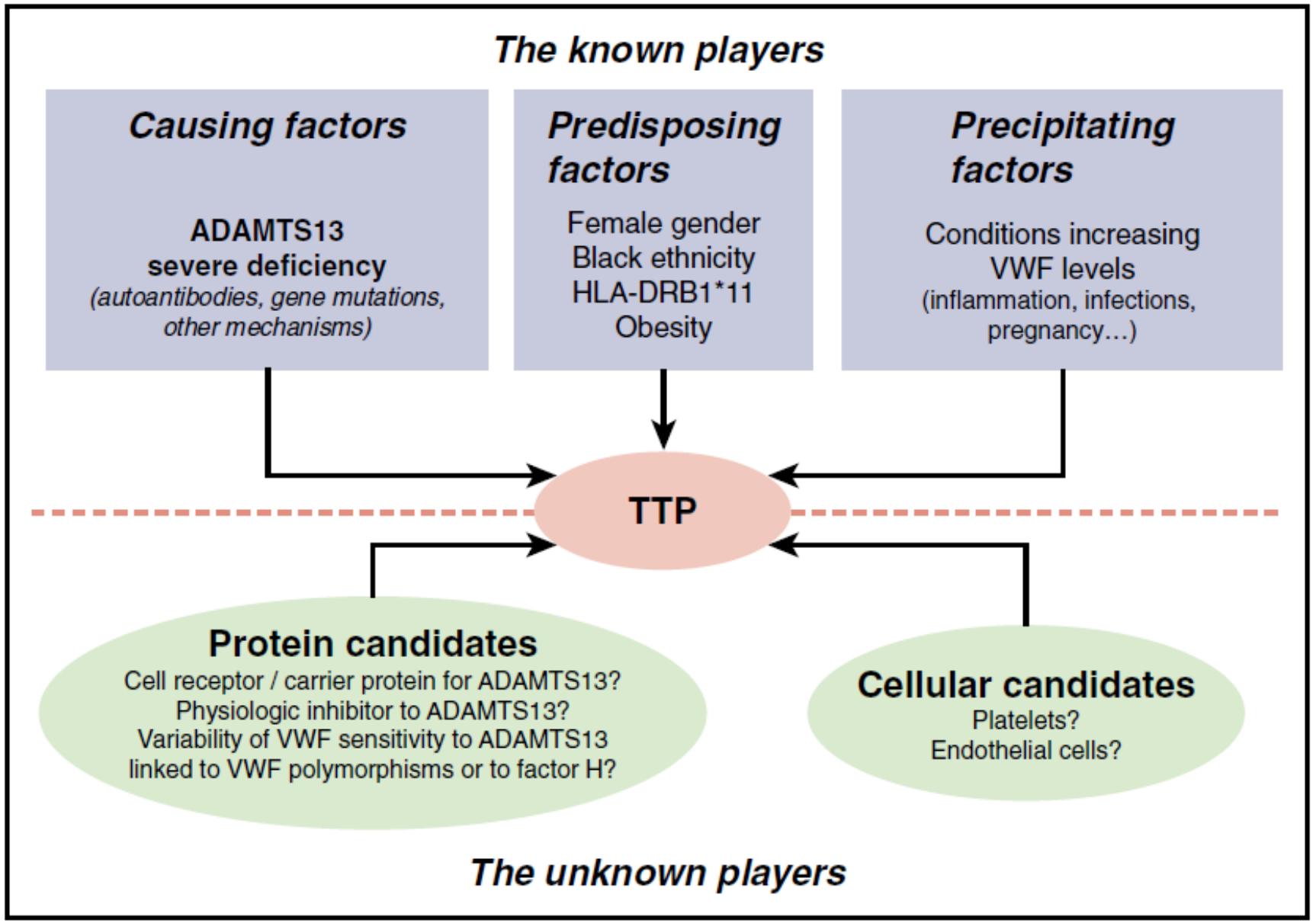
In physiologic conditions, ultralarge VWF multimers released from endothelial cells are cleaved by ADAMTS13 in smaller VWF multimers, less adhesive to plts.

In TTP, because of the absence of functional ADAMTS13, ultralarge VWF multimers are released into the blood and bind spontaneously to plts through GPIb to form aggregates within the arterial and capillary microvessels.

The VWF–platelet aggregates are large enough to form microthrombi inducing tissue ischemia, platelet consumption, and macroangiopathic hemolytic anemia (schistocytes on blood smear).

Known and unknown players involved in TTP.



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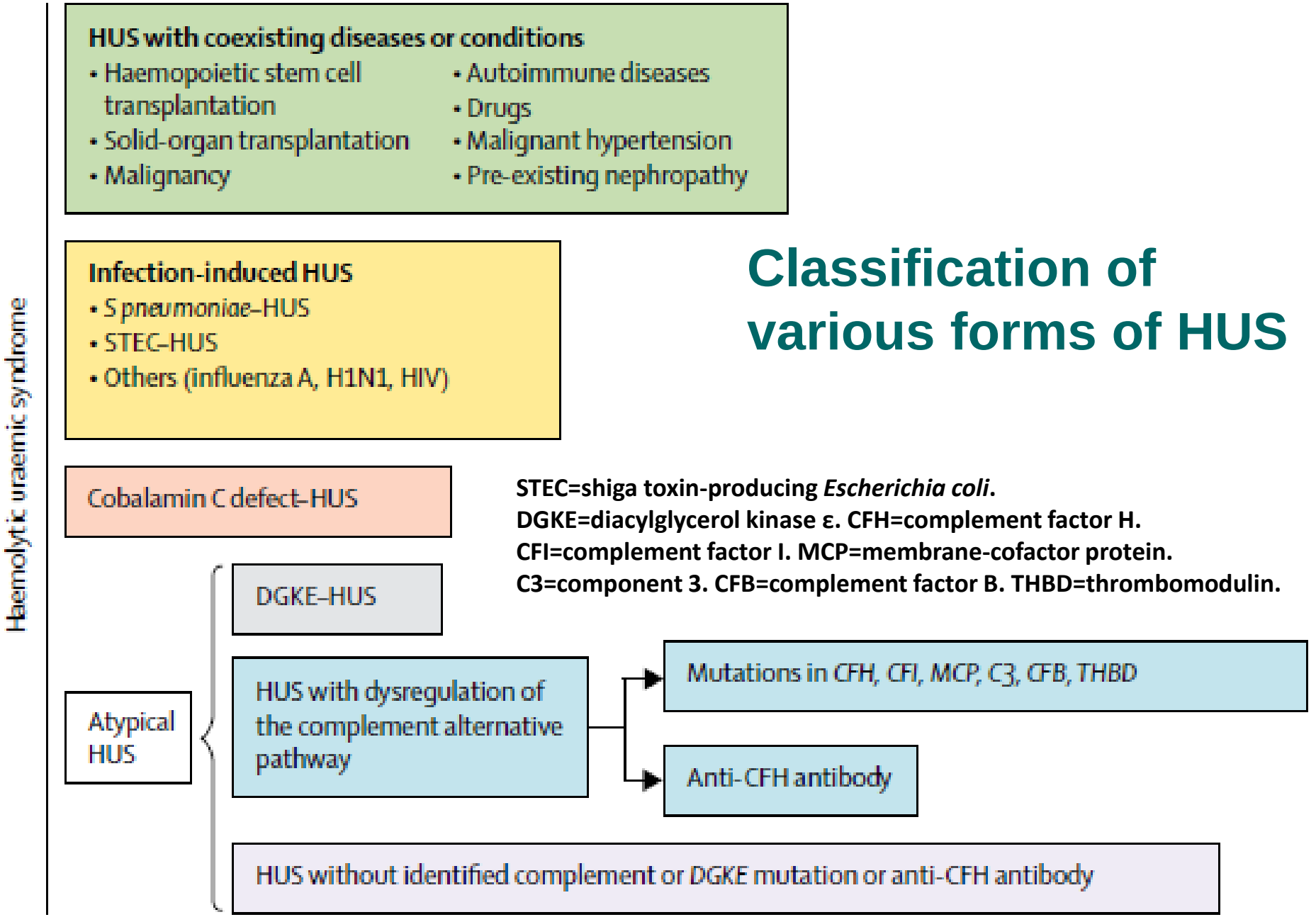
HUS: CLASSIFICATION

- Traditionally, HUS had been divided into diarrhea-positive and diarrhea-negative HUS.

- **Diarrhea positive**, also referred to as **typical HUS**, primarily resulted from Shiga toxin-producing Escherichia coli (STEC) infections, and less frequently from Shigella dysenteriae type 1 infection.

- All other causes of HUS were referred to as **atypical HUS** or assigned to the **diarrhea-negative HUS,** even though some patients with non-STEC-associated HUS also presented with diarrhea.

Classification of various forms of HUS



STEC=shiga toxin-producing *Escherichia coli*.
DGKE=diacylglycerol kinase ε. CFH=complement factor H.
CFI=complement factor I. MCP=membrane-cofactor protein.
C3=component 3. CFB=complement factor B. THBD=thrombomodulin.

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HUS: EPIDEMIOLOGY

- **STEC-HUS occurs primarily in children younger than 5 years of age and in the elderly.**

- **It is difficult to assess the annual incidence of STEC-HUS,**
  - **The overall rates is 2 per 100 000 for all age groups**
  - **Up to 6 per 100 000 in children <5 years.**

- **Many strains of E. coli have been associated with clinical disease including sorbitol non-fermenting and fermenting E. coli O157 as well as E. coli O26, O103, O111 and O145.**
- **E. coli O104:H4 was the specific strain isolated during the large German outbreak in 2011.**

HUS: EPIDEMIOLOGY

- After an incubation period of 4–7 days, STEC-infected patients develop diarrhea and approximately 15% of cases develop HUS within an additional 2–10 days.
- Pts may be infected by intake of
  - contaminated food including raw, processed or undercooked meat, vegetables,
  - unpasteurized juice or milk products,
  - cross-contamination of food products and utensils,
  - intake of contaminated water, even from swimming pools,
  - person-to-person transmission or contact with animals bearing the strain.

HUS: EPIDEMIOLOGY

- Transmission occurs more often in summer, requires a very low number of bacterial organisms and occurs in outbreaks or sporadically.
- Very large outbreaks have occurred in Japan and in Germany.
- In countries in which intake of raw meat is higher, STEC infection is endemic and HUS rates are thus higher, such as in Argentina.

aHUS: EPIDEMIOLOGY

- aHUS is an ultra-rare disease with an estimated incidence that is most probably between 0.5 and 2 per million.
- Onset may occur at any age but is more frequent in childhood particularly before the age of 2 years.
  - Onset before 6 months of age is highly indicative of aHUS as STEC-associated HUS is uncommon in this age group.
- The onset is usually triggered by a febrile infection in the respiratory or gastrointestinal tract.
- Pts who do not develop end-stage renal failure during the first episode tend to relapse, and the disease may affect several members of the same family.

Shiga toxin–producing Escherichia coli infection HUS (STEC-HUS): pathogenesis

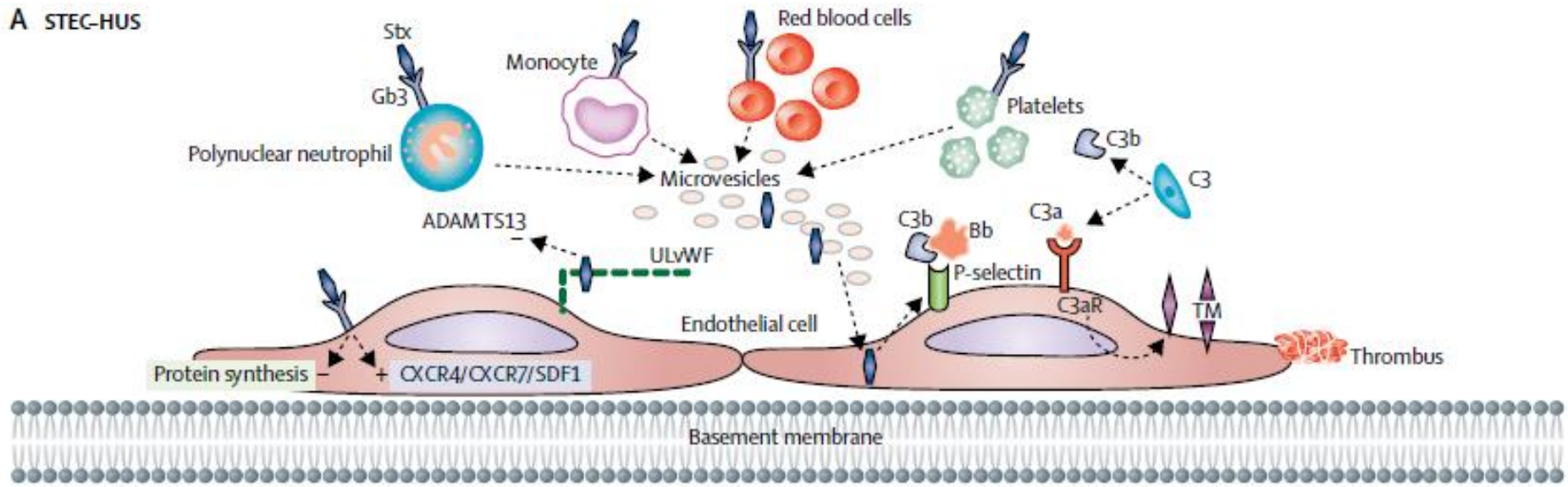
- **The process is apparently initiated when the Shiga toxin (or Shiga-like toxin), a known potent cytotoxin, binds to cell membrane glycolipid Gb3 (via domain B).**

- **Domain A is internalized and subsequently halts protein synthesis and induces apoptosis of the affected cell.**

Shiga toxin–producing Escherichia coli infection HUS (STEC-HUS): pathogenesis

- **The Shiga toxin has several additional effects on endothelial cells, one of which is enhanced expression of functional tissue factor that could contribute to microvascular thrombosis.**
- **The toxin causes damage to or activation of endothelium, red cells, and platelets.**
- **The main reason why gastrointestinal infection particularly affects kidneys is thought to be the tissue tropism of Shiga-toxin on the basis of the strong expression of Gb3 on the glomerular endothelium.**

STEC-HUS



Stx enters the endothelial cell (EC) via Gb3-dependent and Gb3-independent pathways, and exerts its cytotoxic effect via protein synthesis inhibition and enhancement of the CXCR4/CXCR7/SDF1 pathway.

Stx also induces the translocation of P-selectin to the EC surface, favouring the assembly of alternative C3 convertase, the release of C3a, and TM shedding

Stx=shiga toxin. Gb3=globotriaosylceramide 3. TM=thrombomodulin. C3=component 3. CFB=complement factor B. vWF=von Willebrand factor.

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Secondary HUS: pathogenesis

- Secondary HUS is initiated by a coexisting disease or condition.
  - **<u>Infections</u> represent** he most frequently reported diseases that lead to clinically evident secondary HUS.
    - They include, especially those caused by Streptococcus pneumoniae, and the influenza virus.
  - secondary HUS may be associated with **transplantation** (solid organ or bone marrow), **autoimmune disease, cancer, pregnancy, and the use of certain cytotoxic drugs**.

  - Coexisting diseases or conditions may
    - **cause direct cell damage,**
    - **promote activation of the complement in general,**
    - **enhance activation of complement on self cells**

aHUS: pathogenesis

- According to the currently prevailing classification, aHUS is not associated with infections or a coexisting disease.
- It is usually associated with **a genetic or acquired defect in regulation of complement activation on host cells**.
- In a number of aHUS patients, an infection (often an upper respiratory tract infection) precedes the clinical triad typical for TMAs.
- Infections are usually considered as triggers, not as causes of the disease as such.

Frequencies of the most common mutations identified in aHUS patients

Mutated gene/protein	Type	Frequency (%)*	Death or end-stage renal disease 3-10 y after onset (%)†
Factor H (including *CFH/CFHR1* hybrid genes)	Loss of complement regulation	24-28	70-80
MCP (CD46)	Loss of complement regulation	5-9‡	<20
Factor I	Loss of complement regulation	4-8	60-70
C3	Gain of complement activation	2-8	60-70
Factor B	Gain of complement activation	0-4	70
Thrombomodulin	Possibly loss of complement regulation and procoagulative state	0-5	50-60
CFHR1/3 deficiency with anti-factor H autoantibodies	Loss of complement regulation	3-10§	30-70
Diacylglycerol kinase ε	Prothrombotic	0-3‖	46
None identified		30-48	50

*Frequencies of the genetic abnormalities have been adopted from a recent review[8] and cohort studies.[65,66]

†The values represent averages of the earlier reported values.[10,90,65-67]

‡Frequency of the isolated heterozygous *MCP* (CD46) mutation is usually 7% to 8%, but the mutations are frequently found in combination with other mutations in complement genes (up to 22%).[67]

§Autoantibodies against factor H have been reported in 56% of pediatric aHUS cases in India.[68]

‖Diacylglycerol kinase ε mutations are most frequently found in patients with disease manifestation within the first year of life (5%-27% in this population).

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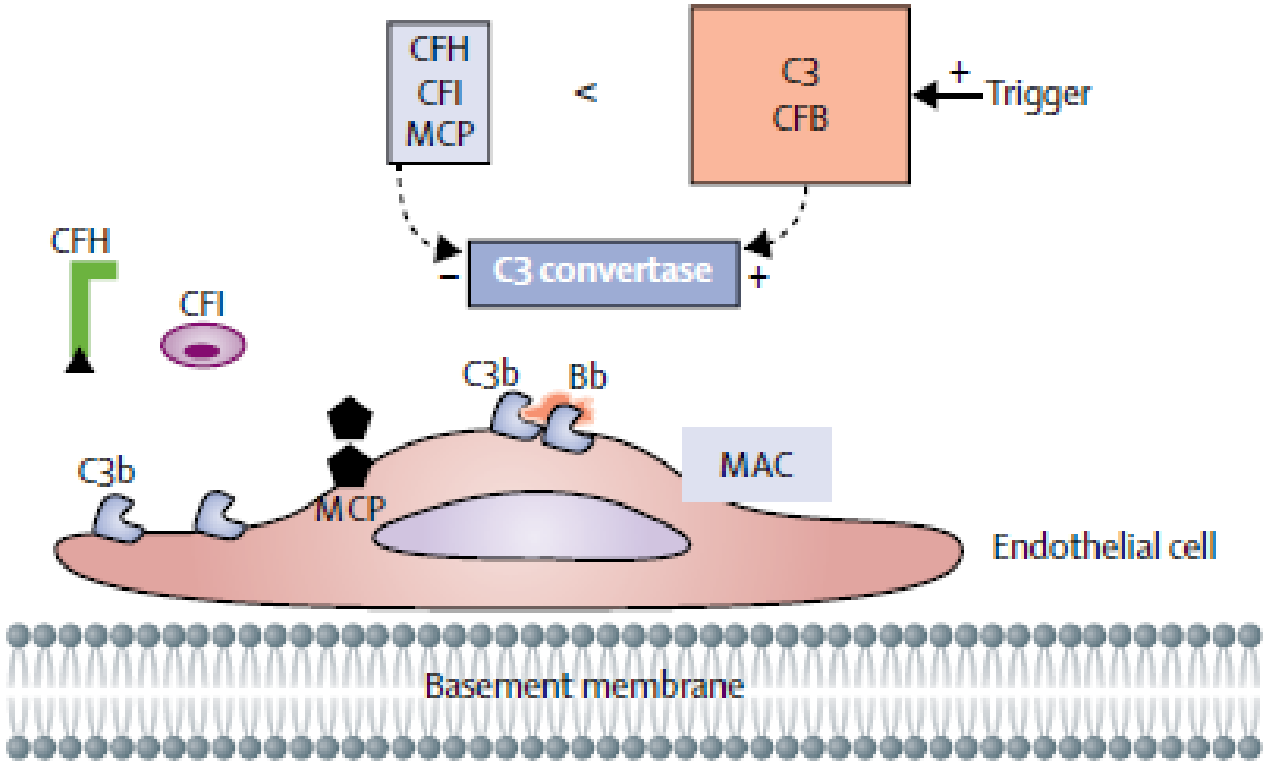
aHUS



The loss of the inhibitory effect of CFH, CFI, or MCP (from inactivating mutations or anti-CFH antibodies) results in the loss of EC protection from CAP-induced damage.

Similarly, gain-of-function mutations in the genes coding for C3 and CFB, the two main components of the alternative C3 convertase, are associated with excessive activation of CAP, resulting in the EC acquiring a procoagulant and proinflammatory phenotype that triggers thrombosis

CAP=complement alternative pathway. CFH=complement factor H. CFI=complement factor I. MCP=membrane-cofactor protein. MAC=membrane-attack complex. C3=component 3. CFB=complement factor B..

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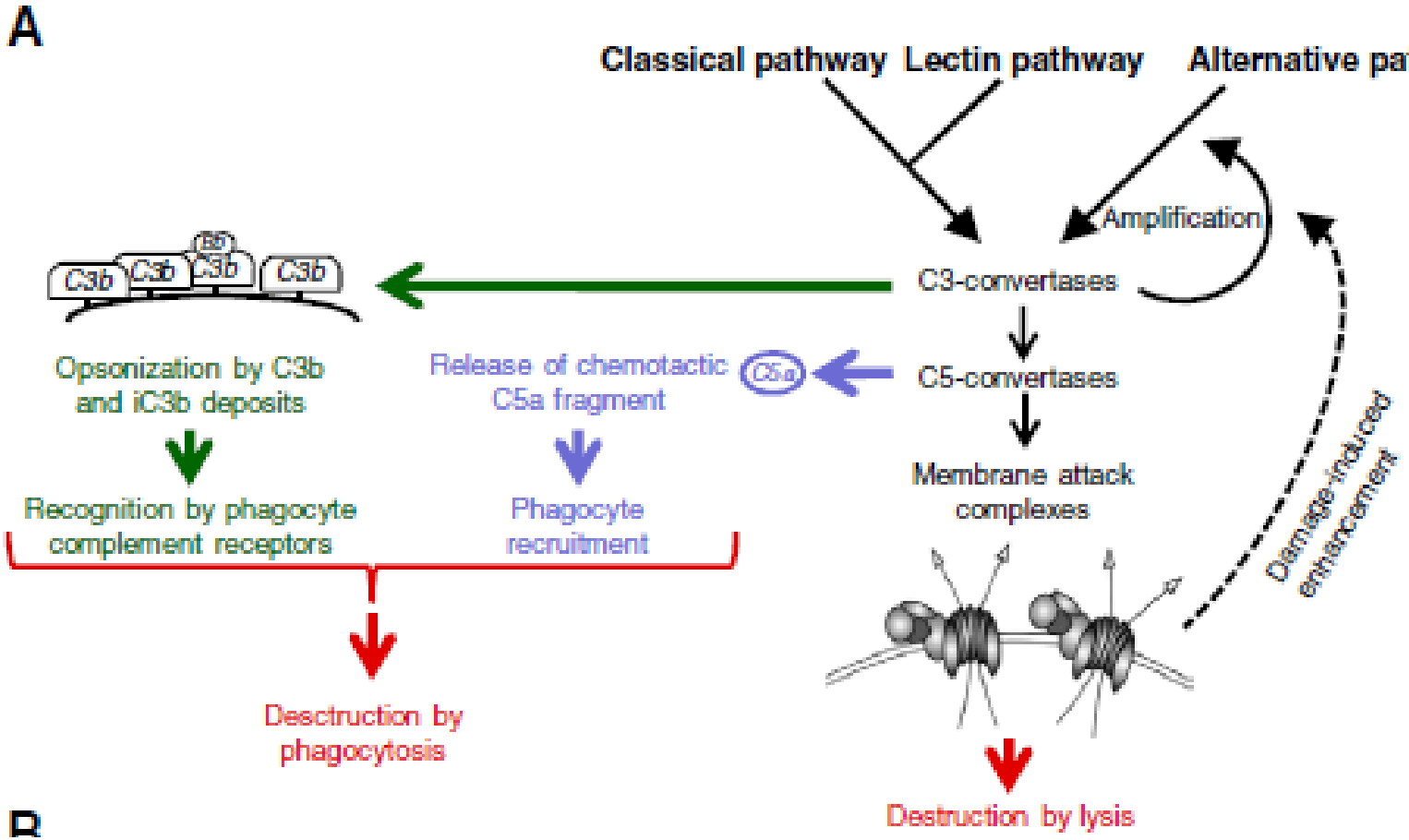
Consequences of complement activation



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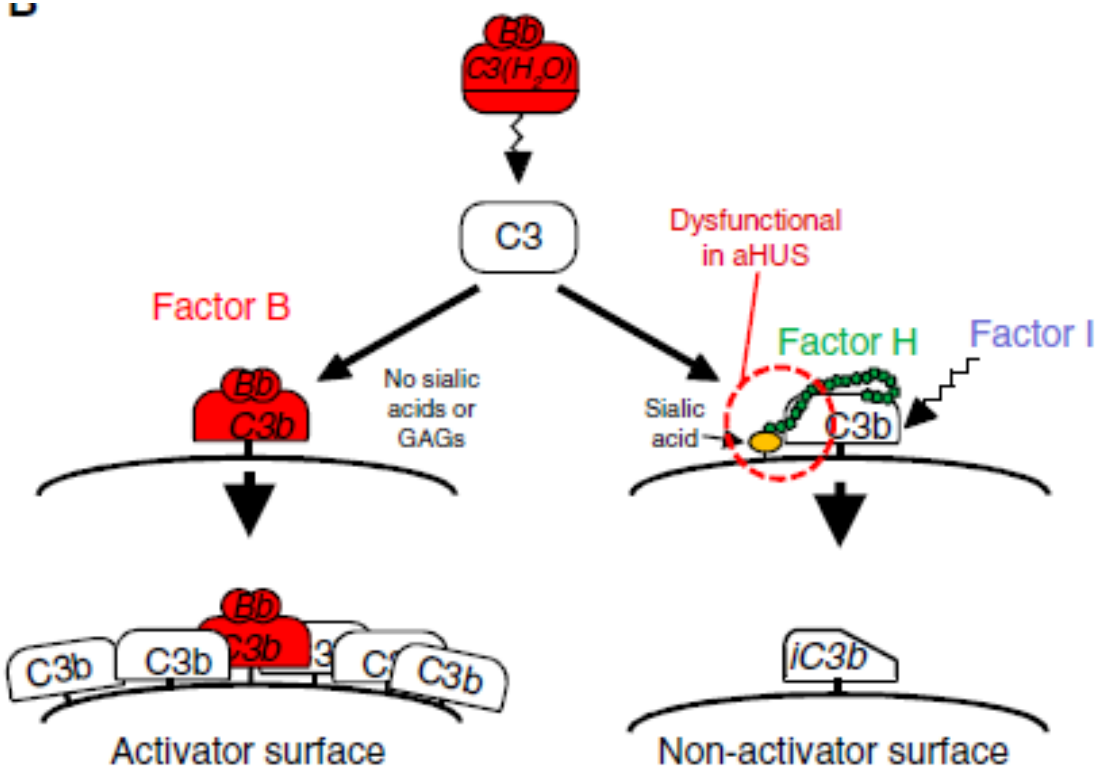
The complement system can be activated via 3 pathways: classical, lectin, and alternative.
All pathways lead to formation of powerful enzymes, the C3-convertases, followed by activation of the terminal cascade.
The main effector functions of complement aim to destroy harmful agents such as microbes.
Lysis of target cells can lead to damage induced enhancement of complement activation.

Consequences of complement activation



iC3b, C3b molecule incapable of forming an enzyme with factor B.

Alternative pathway activation is based on continuous, low-level covalent deposition of C3b molecules onto practically all surfaces in contact with plasma. If the C3b molecule is allowed to form an enzyme (shown in red), new C3b deposits will be formed around the enzyme leading to rapid amplification of the activation.

If the regulator factor H binds to C3b, the convertase enzyme is inactivated and no complement activation follows.

The simultaneous interaction of factor H with both C3b and cell surface sialic acids (or possibly glycosaminoglycans [GAGs]) is essential for proper regulation on self red cells, platelets, and EC.

Disbalance between activation and regulation may lead to pathogenesis of atypical HUS.

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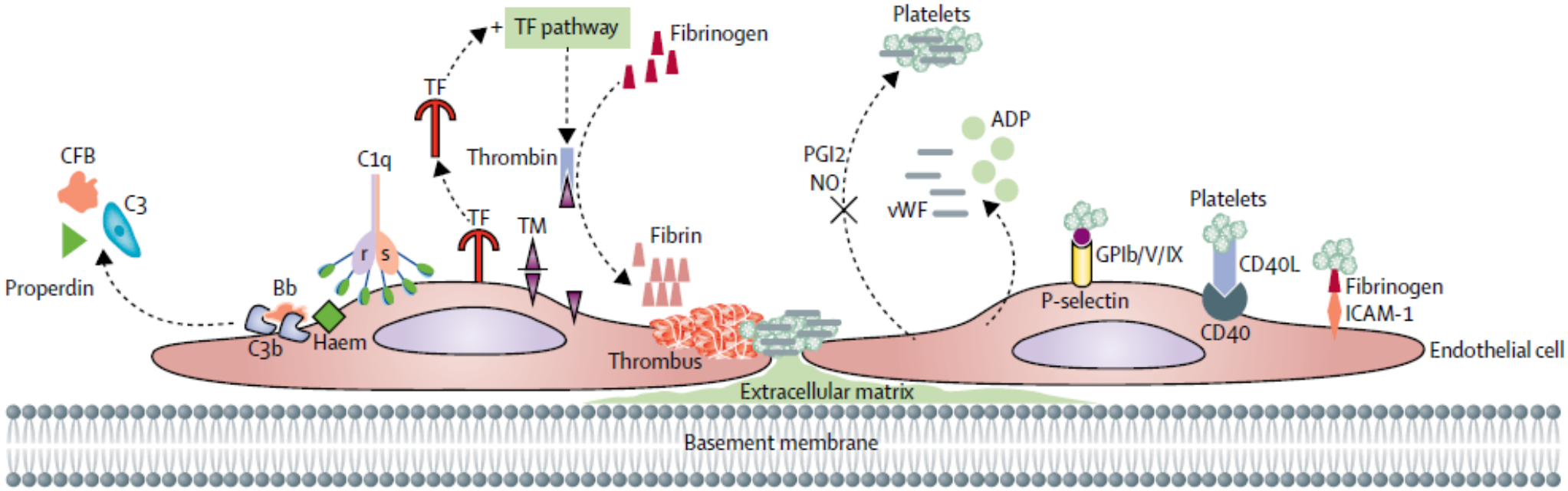
Common final phenotype of endothelial cell in HUS



Distinct initial pathogenic mechanisms of HUS lead to a common final proinflammatory and prothrombotic phenotype of endothelial cells resulting from increased secretion of vWF multimers and ADP, decreased release of NO and PGI2, the upregulation at the endothelial cell surface of various adhesion molecules, expression and secretion of TF, alterations in the glycocalyx, and the shedding of TM.

PGI2=prostacyclin. NO=nitric oxide. TF=tissue factor. TM=thrombomodulin.

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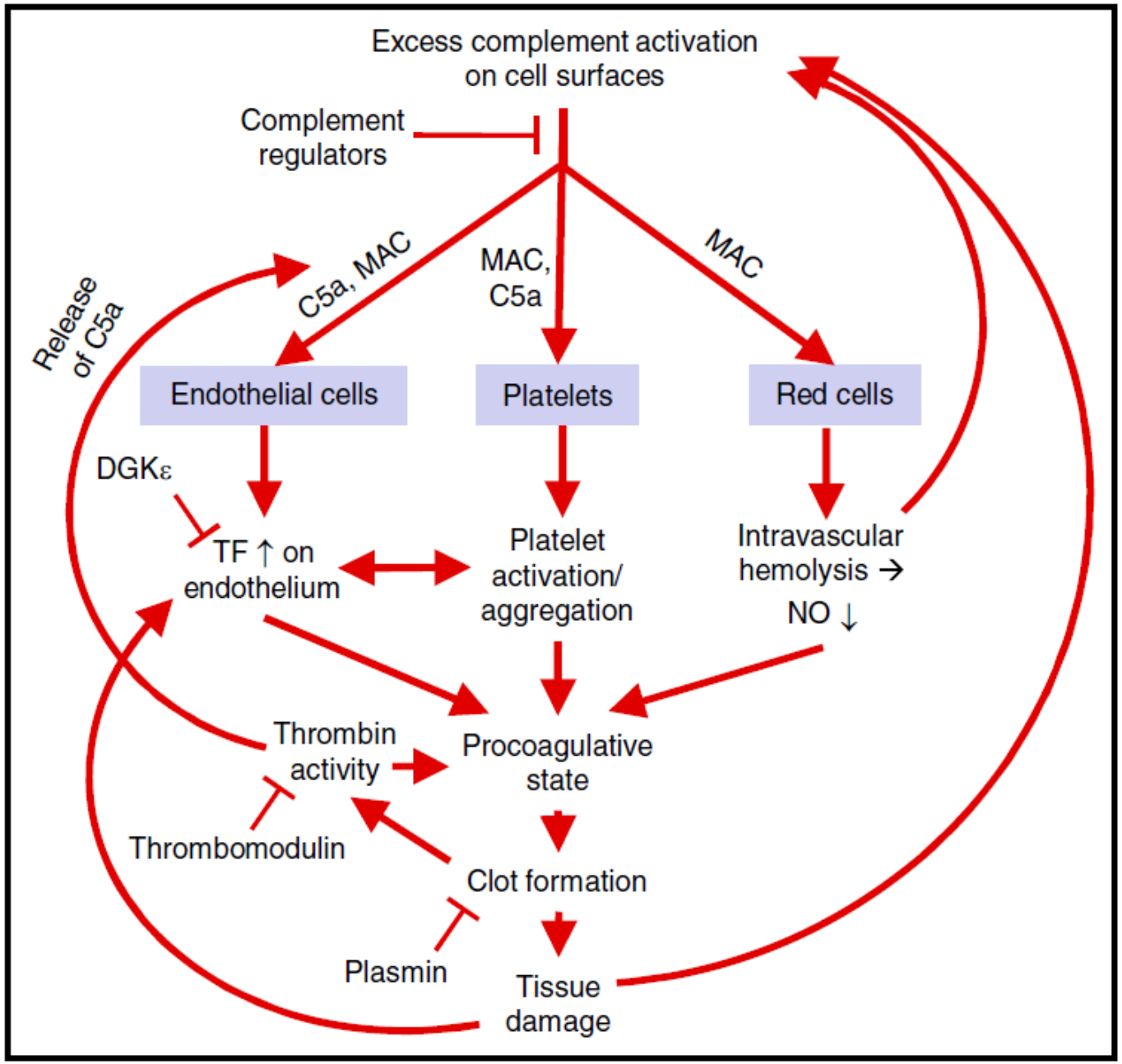

Figure 4. A model of the parallel pathogenic processes in HUS. Excess complement activation on endothelial cell, platelet, and red cell surfaces leads to C5a release and membrane attack complex (MAC) formation. This leads to enhanced tissue factor (TF) activity on the endothelium, activation and aggregation of platelets, and release of hemoglobin and reduction of nitric oxide (NO) in plasma. These phenomena lead to a procoagulative state, coagulation, and thrombosis-mediated tissue damage. There are several feedback loops in this process. These loops can be seen as a cycle that may be initiated at several points and where several phenomena may take place in parallel. This may explain why STEC-HUS, secondary HUS, and aHUS share clinical features although the processes start in various ways. DGKε, diacylglycerol kinase ε.

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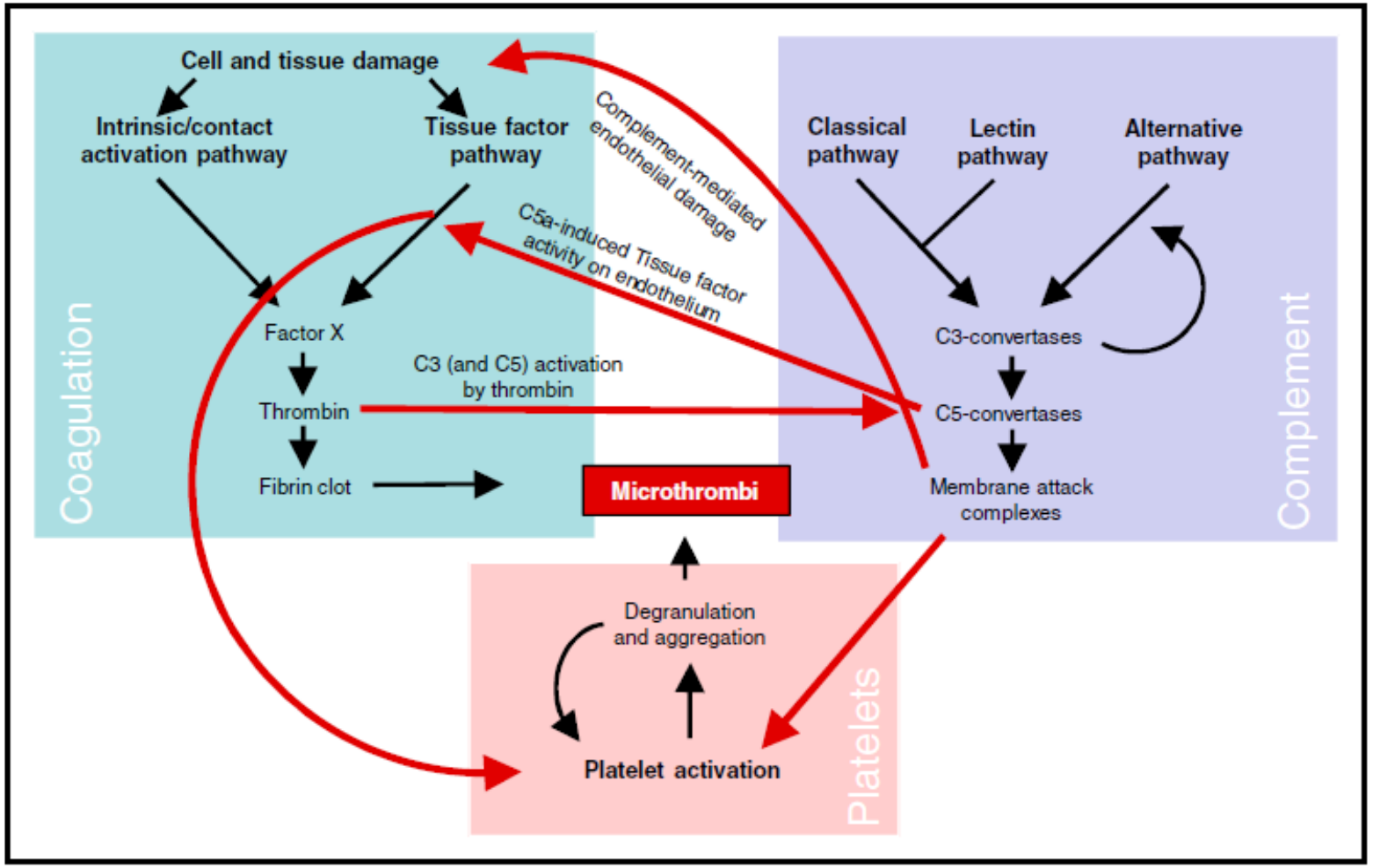
Figure 3. Schematic presentation of the main links between the complement and coagulation systems and platelets in formation of microthrombi in aHUS. Complement activation leads to release of the C5a peptide, inducing tissue factor activity on endothelial cells leading to a procoagulative state of the endothelium. Activation of the coagulation cascade leads to generation of active thrombin that is able to cleave not only fibrinogen but also complement C5, which thereby enables coagulation-enhanced complement activation. Formation of membrane attack complexes on endothelial cells and platelets can cause endothelial cell damage and platelet activation. Finally, activation of the coagulation system leads to platelet activation via various mechanisms. Together, the coagulation system and platelet activation/aggregation lead to formation of microthrombi. The importance of complement in this process in aHUS is clearly demonstrated by rapid inhibition of microvascular thrombosis by therapeutic complement inhibition.



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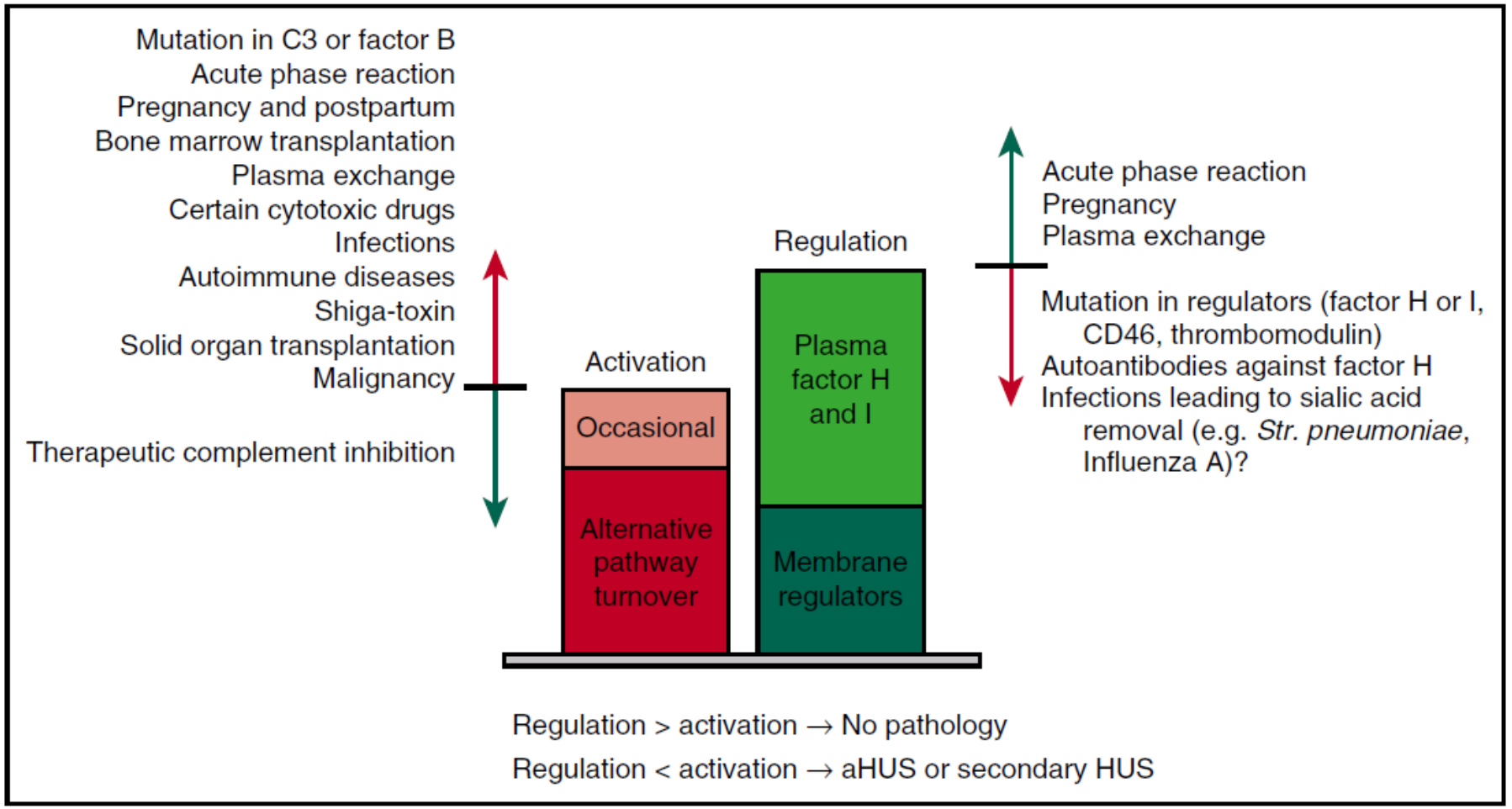
Predisposing factors, promoters, and triggers of aHUS and secondary HUS.



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Common features

- The symptoms and clinical signs may be practically identical in STEC-HUS, secondary HUS, and aHUS.
- Another common feature is that some patients with aHUS and also STEC-HUS and secondary HUS have either mutations or autoantibodies that are associated with impaired complement regulation.
- It is also noteworthy that the same set of cells (endothelial cells, red cells, and platelets) may be damaged or activated in both STEC-HUS and aHUS, and apparently in secondary HUS as well.
- These common features indicate similarities in disease pathogenesis of these 3 forms of HUS.

Sintomatologia TTP

- La TTP acquisita insorge frequentemente in forma acuta, a evoluzione rapida in alcuni gg, con sintomi molto variabili da caso a caso (artralgie, dolori pleurici, fenomeno di Raynaud).

- Possono essere presenti
  - febbre,
  - interessamento neurologico con alterazioni del comportamento, paresi, afasia, in qualche caso con infarto cerebrale da embolizzazione,
  - sintomatologia emorragica mucoso cutanea, raramente d'organo,
  - anemia emolitica con pallore e ittero,
  - interessamento renale fino all'insufficienza renale.

- La sintomatologia addominale da microinfarti viscerali può simulare un addome acuto.

Sintomatologia TTP

- Sono stati distinti due tipi di TTP:
  1. episodio isolato di TTP:
     1. di tipo idiopatico che evolve verso la guarigione (circa due terzi dei pazienti);
  2. TTP ricorrente cronica:
     1. in rari casi, la malattia si presenta con frequenti episodi di varia gravità a intervalli irregolari, richiedenti terapia preventiva.

Le forme familiari di TTP sono molto rare, compaiono nell'infanzia o nella giovinezza e si manifestano a intervalli regolari di circa 3 settimane (TTP familiare ricorrente cronica).

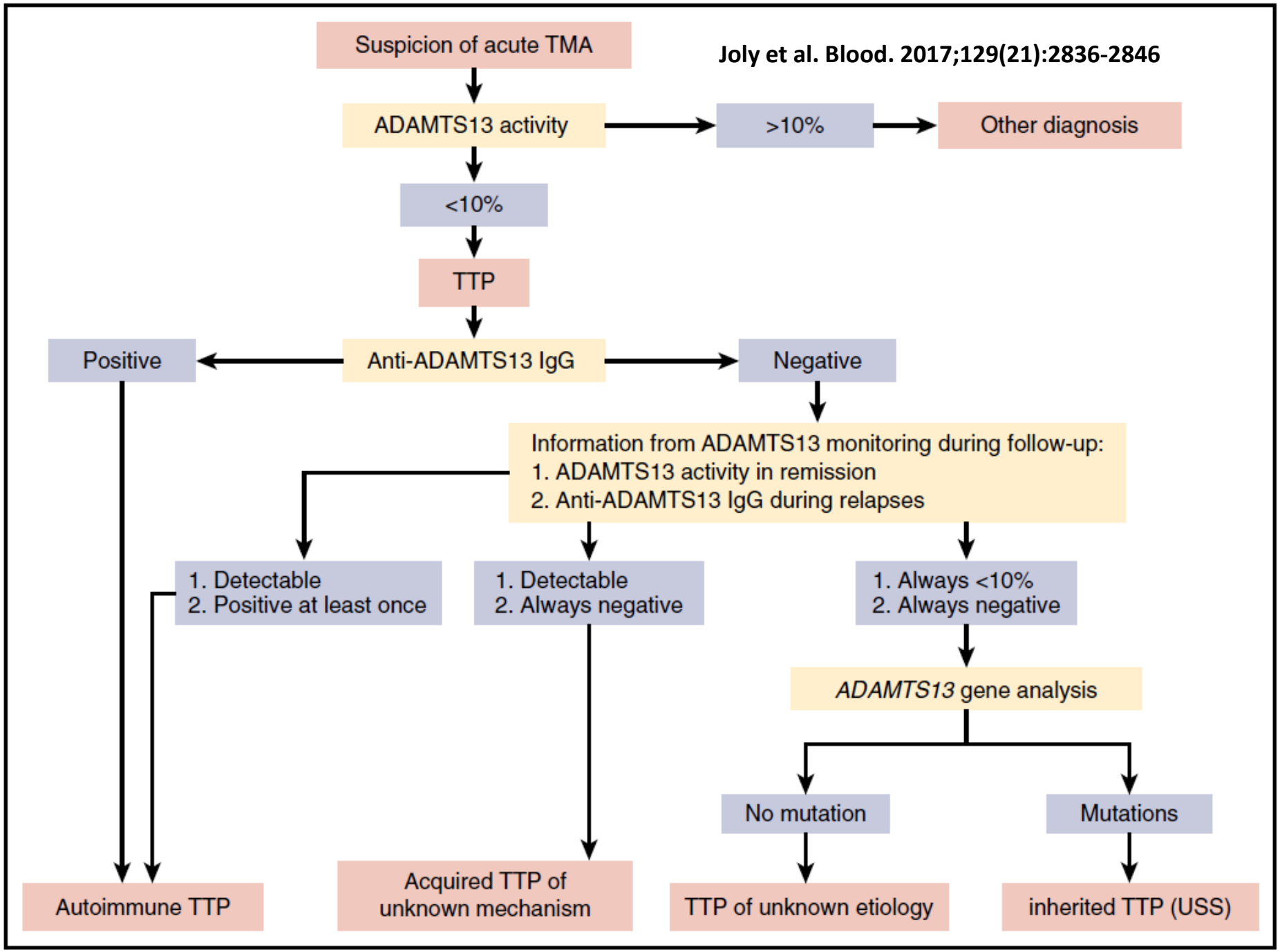
Sintomatologia HUS

- La HUS si sviluppa generalmente nell'infanzia come un singolo episodio di severa insufficienza renale frequentemente preceduto da una gastroenterite con diarrea ematica.

- Le forme familiari di HUS, non sono invece precedute da episodi gastroenteritici essendo causate da una difettiva produzione di fattore H e MCP.

Reperti di laboratorio

- È sempre presente l'anemia, in molti casi grave, con reticolocitosi, presenza di eritrociti nucleati;
- i globuli rossi sono tipicamente e diffusamente frammentati (schistociti);
- la bilirubinemia indiretta e la LDH sono aumentate.
- La piastrinopenia è spesso spiccata, con iperplasia della matrice megacariocitaria midollare.
- La leucocitosi può presentarsi con forme immature, addirittura con aspetti leucemoidi.
- L'azotemia e la creatinina serica sono di solito superiori alla norma.
- Una severa insufficienza renale è caratteristicamente presente nella HUS.
- I test coagulativi sono generalmente nella norma.
- Nella TTP, ma non nella HUS, è possibile dimostrare una ridotta attività di ADAMTS 13 mediante test di laboratorio non ancora però disponibili su larga scala.



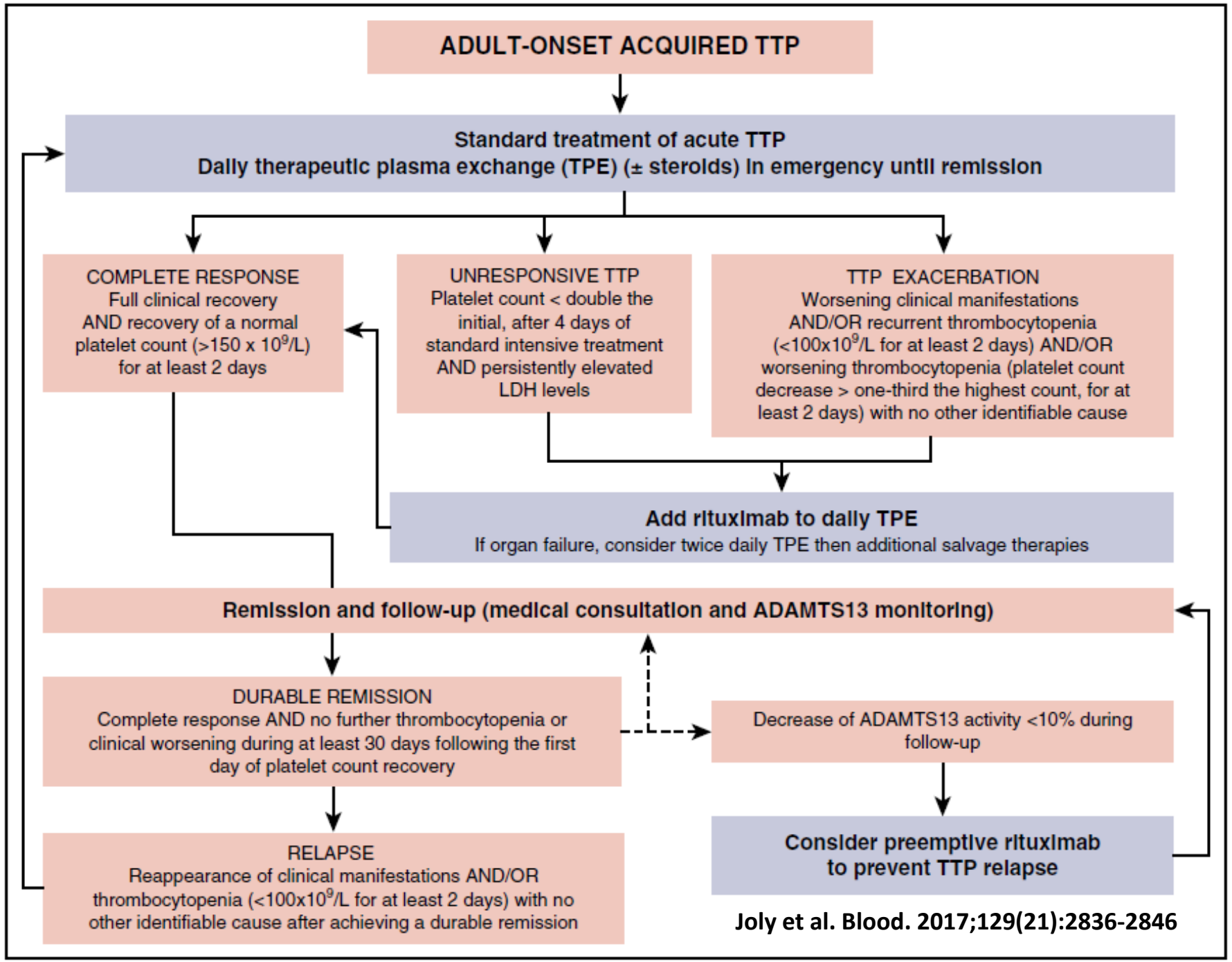
Joly et al. Blood. 2017;129(21):2836-2846

Flowchart for ADAMTS13 investigation in TTP.

Terapia: TTP acquisita

- **Nella TTP acquisita è richiesto il plasma exchange quotidiano.**
  - **Il plasma exchange consiste nella combinazione di plasmaferesi (che rimuove i multimeri ad alto peso molecolare del VWF e gli autoanticorpi contro ADAMTS 13) e l'infusione di plasma fresco congelato (che contiene e pertanto sostituisce l'enzima mancante o danneggiato).**
  - **Alcuni pazienti con elevati titoli di anticorpi anti-ADAMTS 13 non rispondono al plasma exchange.**
    - **Glucorticoidi,**
    - **splenectomia,**
    - **immunosoppressori (CTX, CSA)**
    - **vincristina (depolimerizza i microtubuli piastrinici e altera l'esposizione di recettori sulla superficie delle piastrine),**
    - **Rituximab (anticorpo monoclonale anti CD20).**
  - **In assenza di manifestazioni emorragiche pericolose per la vita del paziente, è prudenziale non ricorrere a trasfusione di piastrine che esacerberebbero la formazione di microtrombi vascolari.**

management of adult-onset acquired TTP.



Joly et al. Blood. 2017;129(21):2836-2846

Terapia: TTP familare/congenita

- Gli episodi di TTP possono essere trattati o prevenuti con l'infusione di plasma fresco congelato, povero di piastrine, contenente ADAMTS 13.
  - In questi casi la plasmaferesi non è richiesta.
  - Non è noto il motivo per cui sono necessarie infusioni ogni tre settimane quando l'emivita plasmatica dell'enzima è di soli due giorni.
  - Livelli di ADAMTS 13 pari al 5% sono sufficienti a prevenire il ricorrere degli episodi acuti di TTP.

HUS: terapia: D+ HUS

- Nei pazienti colpiti da HUS in forma lieve, con oligoanuria della durata inferiore alle 24 ore, è sufficiente un appropriato controllo del bilancio idroelettrolitico.

- Nei restanti casi è necessario ricorrere prontamente al trattamento dialitico. Una breve durata del periodo di anuria e la rapidità nell'iniziare il trattamento dialitico predicono un completo recupero.

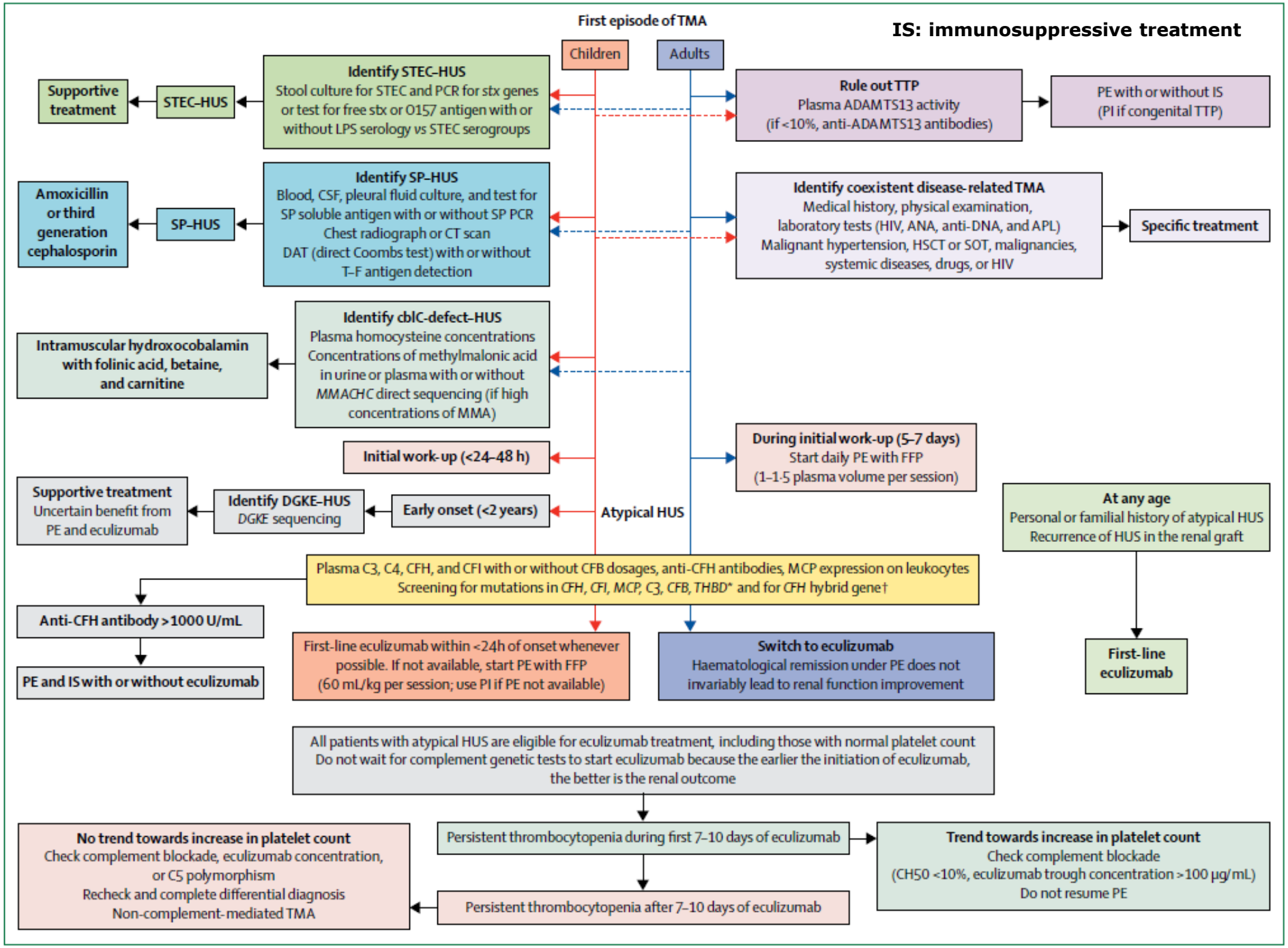
- L'uso di agenti antimicrobici incrementa invece il rilascio della tossina Shiga da parte dell'Escherichia coli O157:H7 e aumenta il rischio di HUS nei bambini infetti.

HUS: terapia D- HUS

- Le forme di Dneg-HUS si possono giovare del plasma exchange o della infusione di plasma fresco congelato: pur tuttavia il 50-75% di questi pazienti progredisce verso un quadro di insufficienza renale che richiede trattamento dialitico.
  - Questa evenienza è invece rara nei casi diarrea positivi.

- I casi di HUS che progrediscono verso l'insufficienza renale possono essere sottoposti a trapianto di rene che tuttavia nelle forme diarrea negative ha una evoluzione sfavorevole nel 50% dei casi.

- Più recentemente è stato proposto Eculizumab: anticorpo monoclonale contro il C5 che blocca la cascata terminale del complemento



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Prognosi

- La prognosi della TTP acuta era molto severa in passato, essendo infausta in oltre il 50% dei casi, ma con le possibilità terapeutiche attuali la sopravvivenza può superare il 90%.

- Le forme di HUS che si presentano con una gastroenterite diarroica hanno un'eccellente prognosi (mortalità pari al 3-5%), mentre le forme familiari non associate a gastroenterite hanno una prognosi molto più severa (mortalità 54%).
- Circa la metà di coloro che sopravvivono presenta recidive e circa un terzo necessita di trattamento dialitico.

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- Sono forme emorragiche complessivamente rare, caratterizzate da prevalenza della porpora cutanea e delle emorragie mucose con tasso piastrinico normale o poco inferiore alla norma, e da difetto dell'emostasi piastrinica con allungamento del tempo di sanguinamento.

- Da un punto di vista patogenetico si distinguono forme da
  - difetto della funzione piastrinica,
  - difetto dei granuli piastrinici,
  - difetto dei recettori e della traduzione del segnale
  - difetto nella esposizione dei fosfolipidi.

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