Jan van Eyck (1434)
Giovanni Arnolfini e sua Moglie Giovanna Cenami
The National Gallery, London

Classificazione patogenetica delle anemie

4. Ridotta sopravvivenza eritrocitaria (emolisi)

- Alterazioni strutturali o metaboliche del GR
- Emolisi immune
- Emolisi meccanica
- Eritrofagocitosi (CpG)

Review
Scand J Infect Dis. 2003;35(9):653-9.
doi: 10.1080/00365540310015999.

CpG DNA: trigger of sepsis, mediator of protection, or both?

Arthur M Krieg

PMID: 14620150 DOI: 10.1080/0036554031001599

Unmethylated CpG motifs are prevalent in bacterial but not vertebrate genomic DNAs and activate immune cells that express the TLR9 receptor. This triggers the production of reactive oxygen species and the secretion of proinflammatory cytokines and chemokines. Under some conditions these effects can result in the systemic inflammatory response syndrome. Under other conditions, the immune stimulatory effects of CpG motifs can protect against pathogen challenge and initiate prophylactic and therapeutic innate and adaptive immune responses.

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

ANEMIA

DNA binding to TLR9 expressed by red blood cells promotes innate immune activation and anemia

L. K. Metthew Lam[1,2], Sophia Murphy[1], Dimitra Kokkinaki[1], Alessandro Venosa[3], Scott Sherrill-Mix[2,4], Carla Casu[5], Stefano Rivella[5], Aaron Weiner[6], Jeongho Park[7], Sunny Shin[4,8], Andrew E. Vaughan[6], Beatrice H. Hahn[2,4], Audrey R. Odom John[9], Nuala J. Meyer[1,2,8], Christopher A. Hunter[7,8], G. Scott Worthen[8,10], Nilam S. Mangalmurti[1,2,8]*

Red blood cells (RBCs) are essential for aerobic respiration through delivery of oxygen to distant tissues. However, RBCs are currently considered immunologically inert, and few, if any, secondary functions of RBCs have been identified. Here, we showed that RBCs serve as critical immune sensors through surface expression of the nucleic acid–sensing Toll-like receptor 9 (TLR9). Mammalian RBCs expressed TLR9 on their surface and bound CpG-containing DNA derived from bacteria, plasmodia, and mitochondria. RBC-bound mitochondrial DNA was increased during human and murine sepsis and pneumonia. In vivo, CpG-carrying RBCs drove accelerated erythrophagocytosis and innate immune activation characterized by increased interferon signaling. Erythroid-specific deletion of TLR9 abrogated erythrophagocytosis and decreased local and systemic cytokine production during CpG-induced inflammation and polymicrobial sepsis. Thus, detection and capture of nucleic acid by TLR9-expressing RBCs regulated red cell clearance and inflammatory cytokine production, demonstrating that RBCs function as immune sentinels during pathologic states. Consistent with these findings, RBC-bound mitochondrial DNA was elevated in individuals with viral pneumonia and sepsis secondary to coronavirus disease 2019 (COVID-19) and associated with anemia and severity of disease. These findings uncover a previously unappreciated role of RBCs as critical players in inflammation distinct from their function in gas transport.

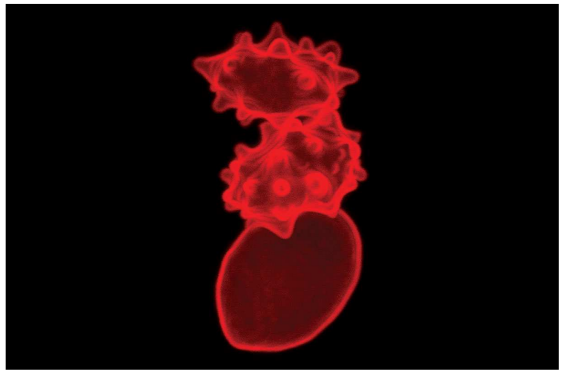
Lam *et al.*, *Sci. Transl. Med.* **13**, eabj1008 (2021) 20 October 2021

Our data demonstrate that RBCs serve as DNA sensors through surface expression of TLR9, which appears to be beneficial during quiescent states, where it promotes scavenging of trace CpG to prevent nonspecific inflammation (*4*). However, during conditions characterized by excess circulating CpG, such as sepsis and COVID-19, binding of CpG by RBC-TLR9 leads to accelerated clearance and inflammation. This innate immune mechanism may be beneficial in the clearance of damaged RBCs and likely contributes to systemic inflammation and the development of anemia during pathologic states where cell-free DNA is elevated. Thus, DNA recognition by TLR9 on RBCs provides bona fide evidence for RBCs as immune sentinels.

CpG induce anche perdita di CD47, un segnale di sopravvivenza degli eritrociti

Red blood cells may be immune sentinels
Oxygen-carrying cells also capture DNA from pathogens and damaged cells

•20 OCT 2021 BY MITCH LESLIE



https://www.science.org/content/article/red-blood-cells-may-be-immune-sentinels

SCIENCE VOL 288 16 JUNE 2000 2051

Role of CD47 as a Marker of Self on Red Blood Cells

Per-Arne Oldenborg,[1] Alex Zheleznyak,[1] Yi-Fu Fang,[1]
Carl F. Lagenaur,[2] Hattie D. Gresham,[3] Frederik P. Lindberg[1]*

The immune system recognizes invaders as foreign because they express determinants that are absent on host cells or because they lack "markers of self" that are normally present. Here we show that CD47 (integrin-associated protein) functions as a marker of self on murine red blood cells. Red blood cells that lacked CD47 were rapidly cleared from the bloodstream by splenic red pulp macrophages. CD47 on normal red blood cells prevented this elimination by binding to the inhibitory receptor signal regulatory protein alpha (SIRPα). Thus, macrophages may use a number of nonspecific activating receptors and rely on the presence or absence of CD47 to distinguish self from foreign. CD47-SIRPα may represent a potential pathway for the control of hemolytic anemia.

Le anemie emolitiche

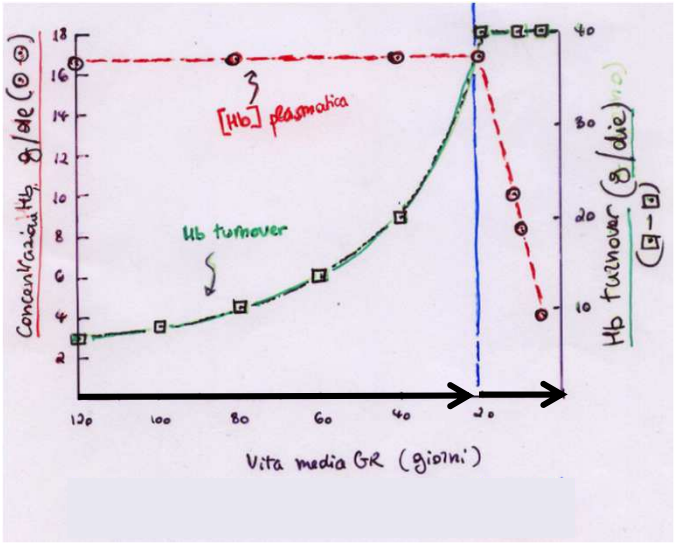
Le anemie emolitiche

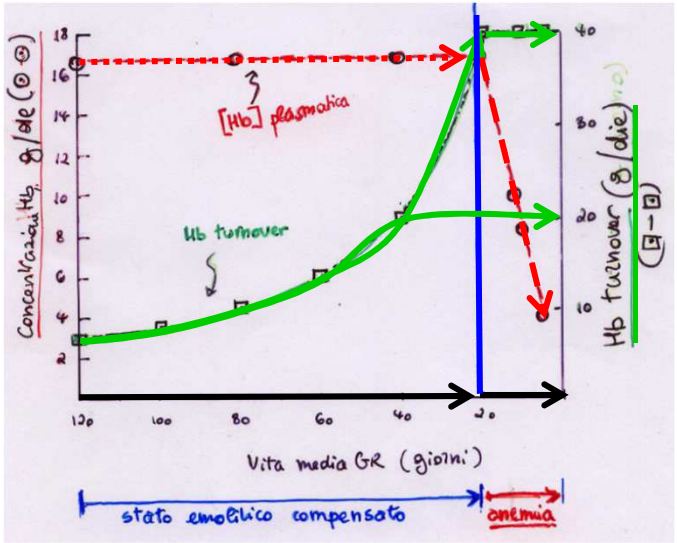
Vita media GR (giorni)	turnover Hb ** (g/giorno)	Hb plasmatica * (g)	[Hb] plasm (g/dl)
120			16.7
80	10.0	800	16.7
40	20.0	800	16.7
20	40.0	800	16.7
12	40.0	480	10.0
10	40.0	400	8.3

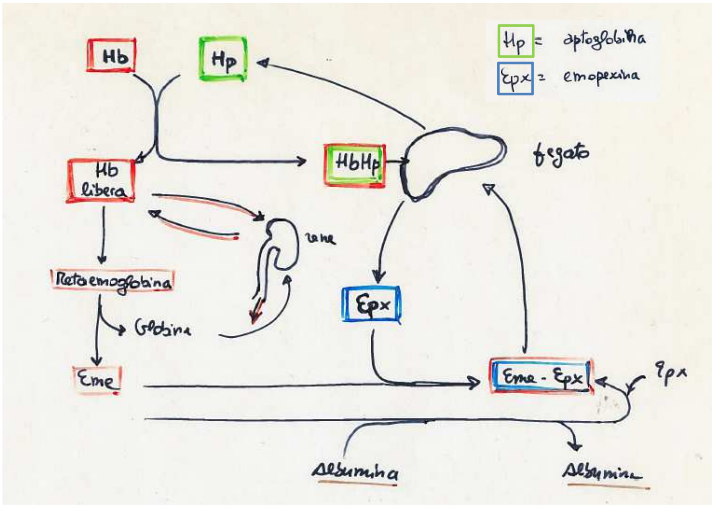
* Assumendo un volume ematico di 4.8 l

** ottenuto da $\frac{\text{Hb totale circolante (g)}}{\text{Vita media GR (giorni)}}$

Ncx Hb turnover = 40g/die (= 6x)







Le anemie emolitiche

- Anemia da aumentata distruzione
  - Ridotta emivita GR
  - Reticolocitosi (risposta alla aumentata distruzione)
  - Bilirubina indiretta aumentata
  - Aumento LDH

Le anemie emolitiche

A. Intracorpuscolari
- Ereditarie (mutazioni germinali)
  - Emoglobinopatie (incluse talassemie e anemia falciforme)
  - Difetti enzimatici
  - Alterazioni del citoscheletro
- Acquisite (mutazioni somatiche)
  - Emoglobinuria parossistica notturna (PNH)

B. Extracorpuscolari
- Fisiche
- Tossiche
- Infettive
- Immuno-mediata
  - Anemia emolitica autoimmune
  - Anemia emolitica alloimmune
- Ipersplenismo

Note sull'anemia falciforme

1. La lesione molecolare: $\beta 6_{Glut \rightarrow Val}$ che comporta diminuita solubilità dopo deossigenazione (HbS)
2. Il meccanismo: i contatti intermolecolari fra catene α e β coinvolgono i residui di superficie 6, 73 e 121 sulle catene β e il residuo 23 sulla α. La Val in posizione $\beta 6$ determina la tendenza di deossi HbS a formare polimeri (6, 8, 14 molecole), strutture bastoncellari che deformano il GR conferendogli la caratteristica forma a falce (sickle)
3. I determinanti:
   - Ossigeno: solo la forma deossigenata polimerizza, processo in certo senso autocatalitico
   - [HbS]: la transizione si ha per [HbS] > 20.8 g/dl
4. Patologia cellulare: la falcizzazione non è un fenomeno istantaneo (nucleazione); sono necessarî circa 2 secondi dopo deossigenazione per cui il tempo di circolo nel capillare è (in genere circa 1 sec) è determinante. L'inizio della falcizzazione rallenta la velocità di transito, aumenta la deossigenazione e crea un circolo vizioso
5. Sintomi: la falcizzazione può generare una crisi vaso-occlusiva a comparsa improvvisa; il dolore acuto può causare infarto e simulare un addome acuto, con variazioni minime dei valori ematologici.

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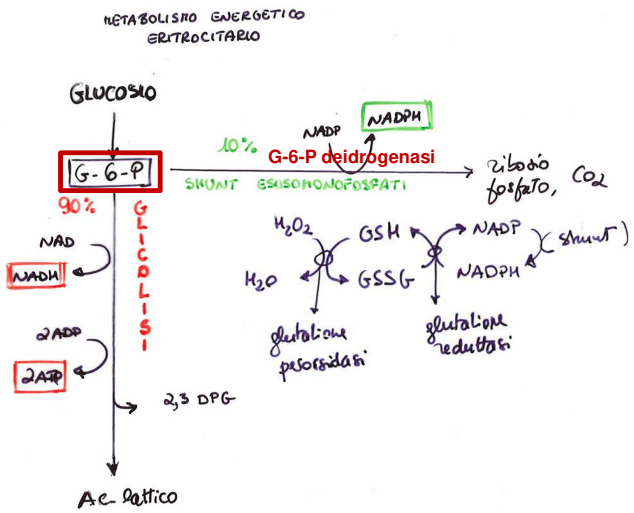


Glucose 6 Phosphate Dehydrogenase (G6PD) Deficiency

Erythrocytes are particularly vulnerable to ROS due to their role in oxygen transport and inability to replace cellular proteins as mature cells. Inherited deficiencies of G6PD can result in acute hemolytic anemia during times of increased ROS production. This may be caused by stress or exposure to certain foods that contain high amounts of oxidative substances, for example, fava beans, or certain medications.

Common medications to be avoided or used with caution in G6PD-deficient patients include:

Acetaminophen, Acetylsalicylic acid, Chloramphenicol, Chloroquine, Colchicine, Diaminodiphenyl sulfone, Diphenhydramine, Glyburide, Isoniazid, L-Dopa, Methylene blue, Nitrofurantoin, Phenazopyridine, Primaquine, Streptomycin, Sulfamides,Trimethoprim, Vitamin K

The *Gd* gene codes for the G6PD enzyme. This gene is located on the **long arm of the X chromosome** and therefore follows X-linked inheritance. Deficiency of G6PD may be due to mutations that change the protein structure and therefore reduce its activity, or the amount of enzyme produced. **There are currently 186 known human G6PD mutations, and most are point mutations affecting a single nucleotide.** None of the mutation patterns seen in humans cause complete inactivation of G6PD since this would be lethal to a developing embryo.

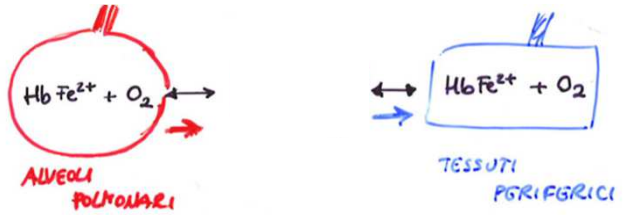
Richardson and O'Malley (2019) StatPearls Publishing LLC

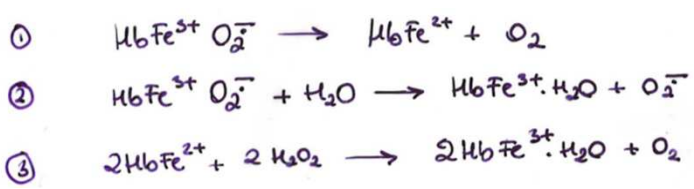
Glucose 6 Phosphate Dehydrogenase (G6PD) Deficiency

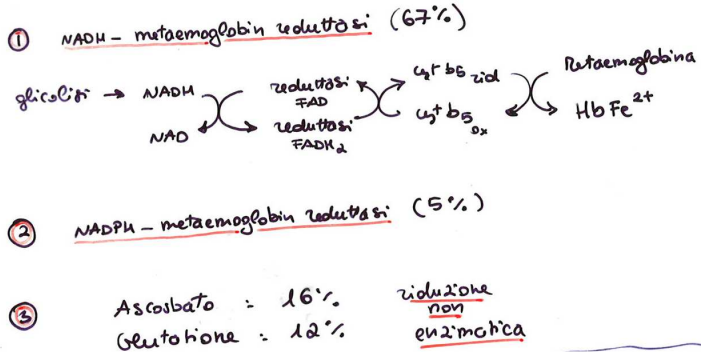
G6PD is the most common human enzyme defect known, affecting upward of 400 million people worldwide. Men are more commonly affected than women due to X-linked inheritance. It is most prevalent in tropical and subtropical areas. Interestingly, **there is evidence to suggest that G6PD deficiency is protective against uncomplicated malaria**, but not severe malaria cases. The protective mechanism for G6PD deficiency and malaria is still being investigated. With regards to ethnicity, **G6PD deficiency is more common in people of African, Mediterranean, or Asian descent, likely owing to its suggested protective effect from malaria.**

G6PD is the catalyst in the rate-limiting first step of the pentose phosphate pathway, which uses glucose-6-phosphate to convert nicotinamide adenine dinucleotide phosphate (NADP) into its reduced form, NADPH. In red blood cells, **NADPH is critical in preventing damage to cellular structures caused by oxygen-free radicals.** It does this by serving as a substrate to the enzyme glutathione reductase. Reduced glutathione can be used to convert hydrogen peroxide to water and prevent damage to cellular structures, particularly the cell wall of red blood cells (RBCs) since they have limited capacity for repair once mature.

Richardson and O'Malley (2019) StatPearls Publishing LLC







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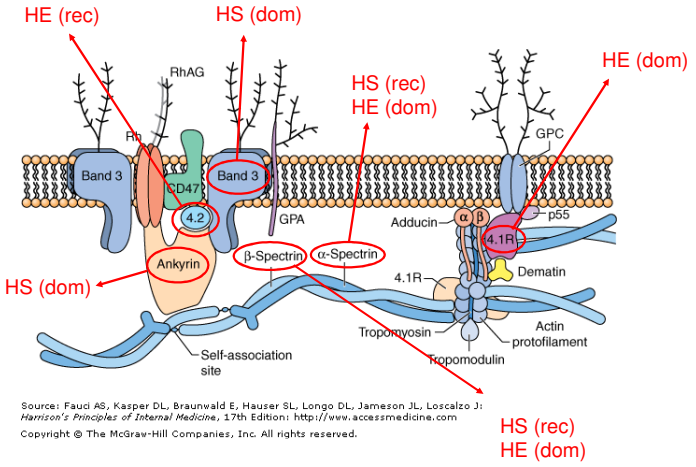
Le anemie emolitiche

Difetti del citoscheletro

- Sferocitosi ereditaria (HS)
  - 1:5.000
  - Quadro clinico variabile
    - Grave anemia emolitica
    - Quadri via via più lievi (a volte scoperta in esami di routine)
- Ellissocitosi ereditaria (HE)
  - Variabilità clinica (da asintomatica a anemia emolitica conclamata), senza correlazione con il quadro morfologico



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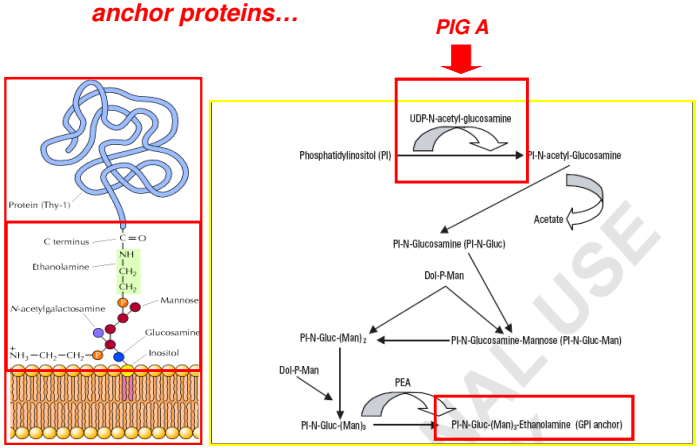
…PIG A e biosintesi delle GPI anchor proteins…



Le cause di anemia emolitica

Emoglobinuria parossistica notturna (PNH)

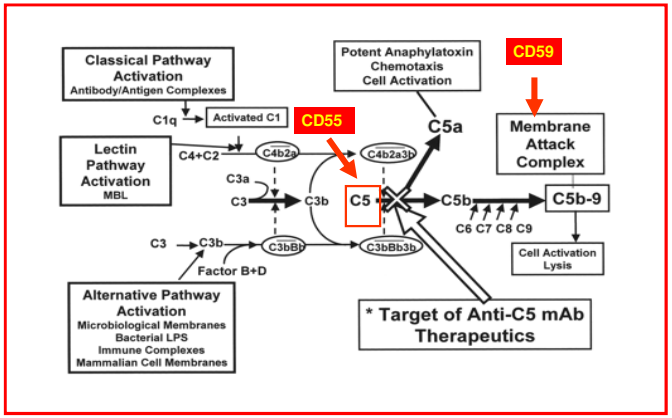
Rara patologia acquisita caratterizzata da un'espansione clonale non maligna di una o più cellule staminali ematopoietiche, causata da mutazioni somatiche a livello del gene PIG A sul cromosoma Xp22

…le Conseguenze….

Antigen	Expressed in	Antigen	Expressed in
Enzymes:		*Blood groups antigens:*	
Acetylcholinesterase	Red cells	Antigen Cromer	Red cells
CD73 (ecto-5'-nucleotidase)	Lymphocytes	Antigen Yt	Red cells
Neutrophil alkaline phosphatase (NAP)	Neutrophils	Antigen Holley Gregory	Red cells
Adhesion molecules:		Antigen John Millton Hagen (JMH)	Red cells, lymphocytes
CD48	Lymphocytes, monocytes	Antigens Dombrock	Red cells
CD58 (LFA-3)	Lymphocytes, red cells	*Other membrane antigens:*	
CD67	Neutrophils, eosinophils	CD52 (CAMPATH antigen)	Monocytes, lymphocytes
CD66	Neutrophils, eosinophils	CD24 (Blast-1)	Neutrophils mainly, lymphocytes
Complement regulatory proteins:		NB1/NB2	Neutrophils
CD55 (DAF)	All hematopoietic lineages	CD157	Neutrophils, monocytes
HRF60 (C8 binding protein)	All hematopoietic lineages	CD90 (Thy-1)	Lymphocytes
CD59 (MIRL)	All hematopoietic lineages	GP500	Platelets
Receptors:		GP175	Platelets
CD16 (FC□III)	Neutrophils, T-lymphocytes	CD90	Some hematopoietic stem cells
CD14	Monocytes	CD109	Hematopoietic stem cells
CD87 (u-PAR)	Neutrophils, platelets, T- and NK-cells		

[5]

…CD 55 e CD 59…



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FENOMENI TROMBOTICI [5]

1: Exp Hematol. 2008 Dec;36(12):1616-24. Epub 2008 Oct 26.
Related Articles, Links

Increased soluble urokinase plasminogen activator receptor (suPAR) is associated with thrombosis and inhibition of plasmin generation in paroxysmal nocturnal hemoglobinuria (PNH) patients.

Sloand EM, **Pfannes L**, **Scheinberg P**, **More K**, **Wu CO**, **Horne M**, **Young NS**.

National Heart Lung and Blood Institute, Hematology Branch, Office of Biostatistics Research, Division of Prevention and Population Sciences, National Institutes of Health, Bethesda, MD 20892-1260, USA.
Sloande@nhlbi.nih.gov

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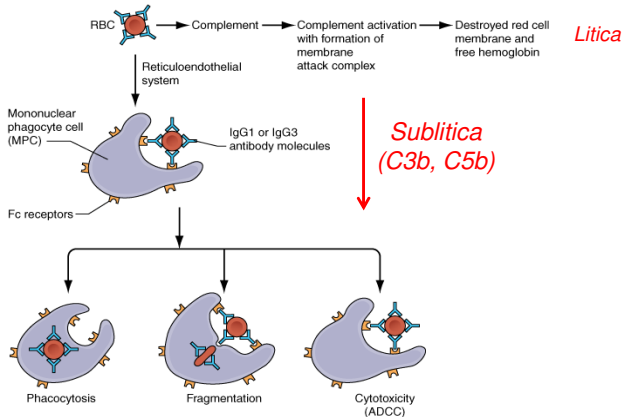
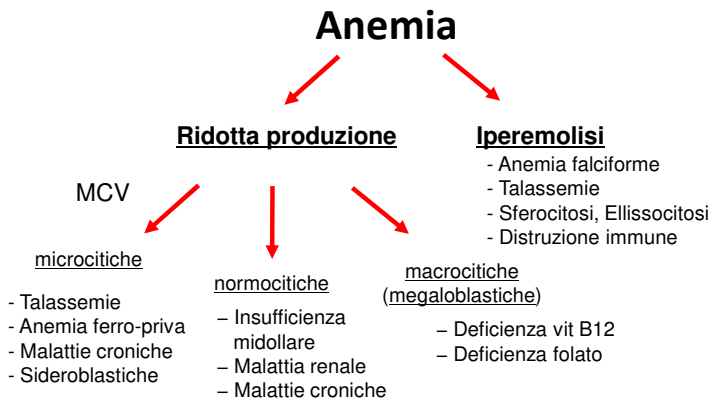
- Cause fisiche
  - Calore (ustioni)
  - Trauma meccanico
    - emoglobinuria da marcia
    - Protesi valvolari
    - Anemia emolitica microangiopatica
- Cause tossiche
  - Veleni di ragni e serpenti (fosfolipasi)
  - Arsenico, alcool
- Cause infettive
  - Malaria
  - *E. coli* 0157:H7 (Shiga toxin, HUS)
  - C. perfringens (lecitinasi)
- Ipersplenismo
  - Ipertensione portale
  - Malattie linfoproliferative

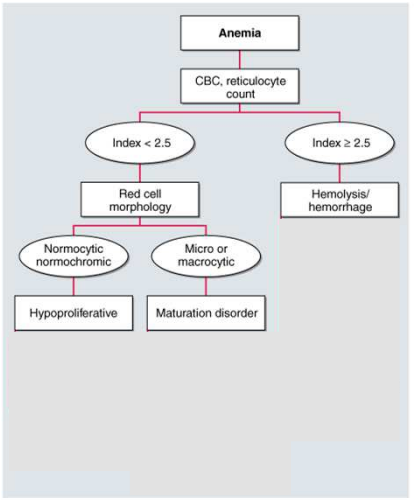
In caso di porpora trombotica trombocitopenica con distruzione non immunologica delle piastrine complicata da sindrome emolitico-uremica (5-10% dei casi). Danno endoteliale con deposito diffuso di filamenti composti di piastrine e fibrina nei piccoli vasi e danno meccanico di piastrine e globuli rossi che li attraversano, con conseguente grave trombocitopenia e anemia (**anemia emolitica microangiopatica**). Le piastrine vengono consumate anche per la formazione di micro-trombi diffusi, contribuendo alla trombocitopenia.

- Alloimmune
  - Trasfusionale (shock distributivo)
  - Malattia emolitica del neonato (incompatibilità Rh)
- Autoimmune
  - Anticorpi caldi (IgG), normalmente fissano il complemento fino a C3 ➔ opsonizzazione ed emolisi extravascolare
    - Primaria 45%
    - Secondaria 40%
      - Malattie linfoproliferative
      - Malattie autoimmuni
      - Malattie infettive
    - Indotta da farmaci 15%
      - Da immunocomplessi (es chinino)
      - Aptene (es penicillina)
      - Risposta autoimmune a prot. membrana (es metildopa)
  - Anticorpi freddi (IgM), legano Ag aT inferiori (<30° C) e fissano l'intera cascata del complemento ➔ emolisi intravascolare
    - Emoglobinuria parossistica da freddo (Ab Donath-Landsteiner)
    - Malattia delle agglutinine fredde



Antibody-mediated destruction or alteration of a red blood cell (RBC). An effector cell recognizes an RBC by antibodies that are bound to the RBC's cell membrane. Three mechanisms can lead to the RBC's destruction or alteration: (i) An RBC is engulfed by a macrophage (MΦ) and lysed intracellularly (phagocytosis). (ii) An RBC is partially phagocytized (fragmentation), but the altered RBC (spherocytes) escapes the immune attack by the macrophage (MΦ) and remains circulating. (iii) An RBC is attacked by a macrophage (MΦ) and lysed extracellularly (ADCC, antibody-dependent cell-mediated cytotoxicity). Modified from Garratty, *Transfus Med.* 2008;18:321–34.





Source: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, Loscalzo J:
Harrison's Principles of Internal Medicine, 17th Edition: http://www.accessmedicine.com

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ami