Acquired hemolytic, megaloblastic and sideroblastic anemias

Dr Alain Kentos

CH Jolimont and ULB-Hôpital Erasme

BHS Educational Course

Red Blood Cell Disorders

November 2013

Topics

- Acquired hemolytic anemias
  - Autoimmune
  - (Microangiopathic)
  - mechanical
  - Infections
  - (Paroxysmal nocturnal hemoglobinuria)
  - Other
- Acquired megaloblastic anemias
  - Vitamin B12 deficiency
  - Folic acid deficiency
- Acquired sideroblastic anemia

Common causes of acquired extravascular destruction of red cells

- Hypersplenism
- Infections (bartonella bacilliformis, babesia, malaria)
- Lead, copper, snake and spider bites
- Auto-immune hemolytic anemia (warm or cold, drugs)
- Oxydant agents (dapsone, nitrites, …)

Common causes of acquired intravascular hemolysis in adults

- Microangiopathic hemolytic anemia: TTP, HUS, prosthetic valve leak
- Direct trauma (runners)
- Transfusion reactions
- Infection (clostridium perfringens sepsis with degradation of phospholipids of the red cell membrane bilayer and the structural membrane proteins, severe malaria)
- Paroxysmal nocturnal hemoglobinuria
- Liver disease with acquired alterations in red cell membrane
- Cold agglutinin disease / paroxysmal cold hemoglobinuria
- Intravenous infusion with hypotonic solutions
- Snake bites
- Compounds with high oxydant potential (copper poisoning, Wilson disease)
- IV immunoglobulins

Autoimmune hemolytic anemia (AIHA)

- Definition
  - Destruction of red blood cells (hemolysis)
  - Due to autoantibodies
  - With or without complement activation
- Detection of autoantibodies by a positive direct antiglobulin test (DAT) or direct Coombs test
- Rare incidence: 1/100000/year
- A negative direct Coombs does not exclude the diagnosis of AIHA
- Presence of microspherocytes in blood smear may support the suspected diagnosis of AIHA with negative Coombs
- Warm or cold antibody?
  - laboratory criteria
  - optimal temperature for autoantibody binding to RBC

Autoimmune hemolytic anemia (AIHA)

- Normal subjects
- Positive Coombs test in normal blood donors: 1/1000 to 1/36000
- Risk factors: AIDS, drugs, age, elevated IgG, cardiolipin antibodies
- A positive Coombs test may predate a malignancy by months to years

Autoimmune hemolytic anemia (AIHA)

- Importance of isotype

  - IgM are very efficient in complement activation
    - Only one molecule of antibody is needed
  - IgG1 and IgG3 are efficient complement activators
  - IgG2 and IgA are weak complement activators
  - IgG4 does not activate complement

  - Generally, complement system not completely activated

Autoimmune hemolytic anemia (AIHA)

- Importance of isotype

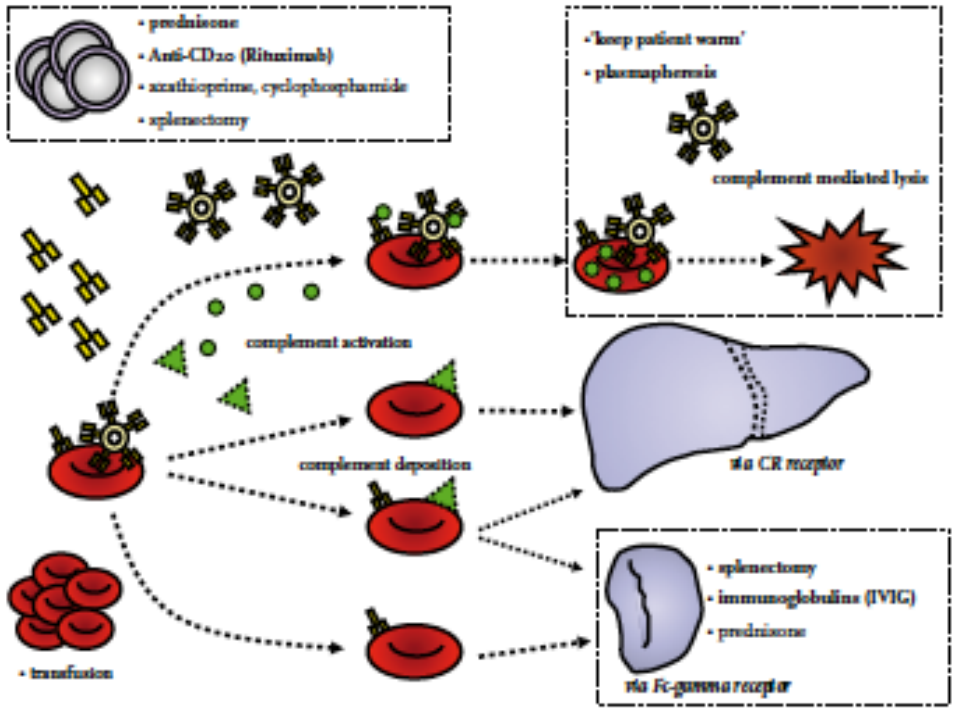
  - Cold antibodies
    - Optimal binding to RBC below 30° C
    - Mostly of IgM isotype
  - Warm antibodies: 75%-80% of the cases
    - Optimal binding at 37° C
    - Mostly IgG, less commonly IgM, rarely IgA
  - Biphasic antibodies
    - Optimal binding below 30° C
    - Induce complement activation at 37° C

Autoimmune hemolytic anemia

- RBC coated with IgG with/without C3/C3d
  - preferentially removed via FC-gamma mediated phagocytosis in the spleen
- RBC coated with C3/C3d in the absence of IgG
  - destroyed via complement-receptor mediated phagocytosis in the liver
- IgM: complement activation possible until formation and introduction of the membrane attack complex leading to intravascular hemolysis

- IgG: 20-60%
- IgG+C3d: 25-65%
- C3d alone: 7-15%
- IgM+C3d: 15%

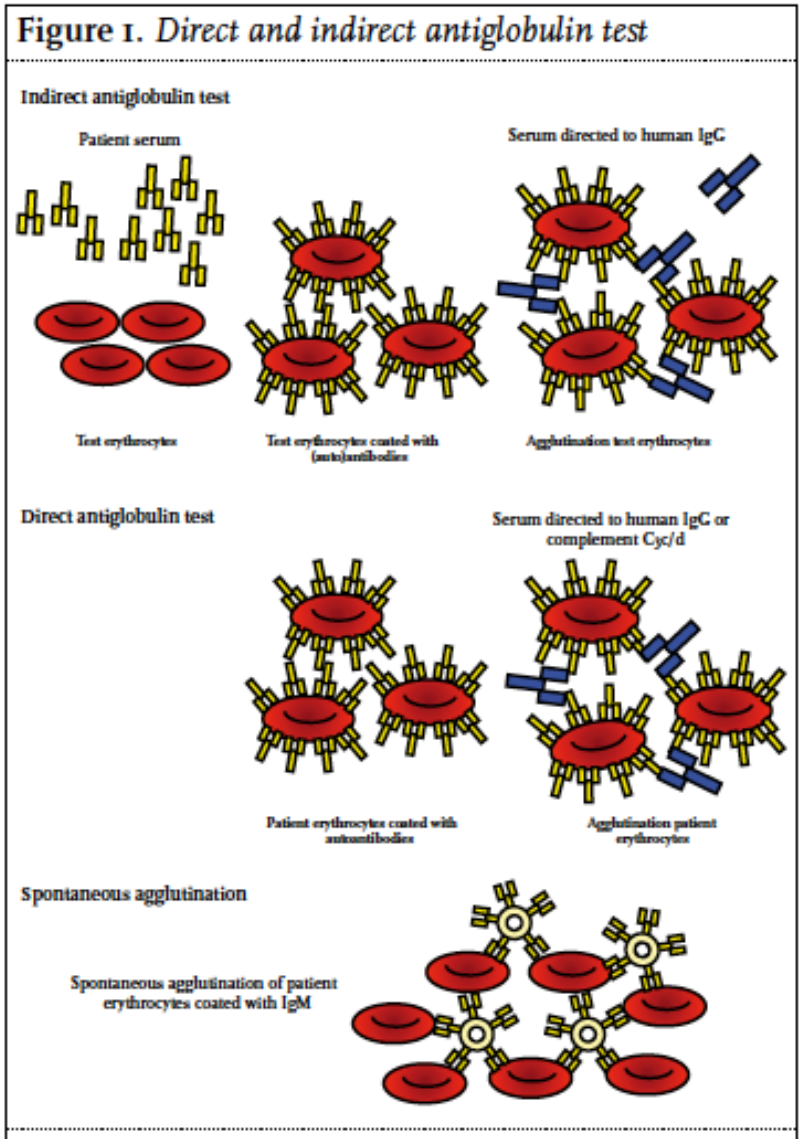
Figure 5. *Mechanisms of red blood cell removal in autoimmune haemolytic anaemia*



Erythrocytes coated with IgG autoantibodies are mainly removed via Fc-gamma receptors on macrophages in the spleen. Complement deposition on erythrocytes in the absence of IgG leads to red blood cell removal in the liver via complement receptors on Kupfer cells. In case of fulminant haemolysis, red blood cells are destructed in the circulation.

Autoimmune hemolytic anemia (AIHA)

Zeerleder S. Autoimmune haemolytic anemia – a practical guide to cope with a diagnostic and therapeutic challenge. The Netherlands Journal of Medicine 2011;69:177-184

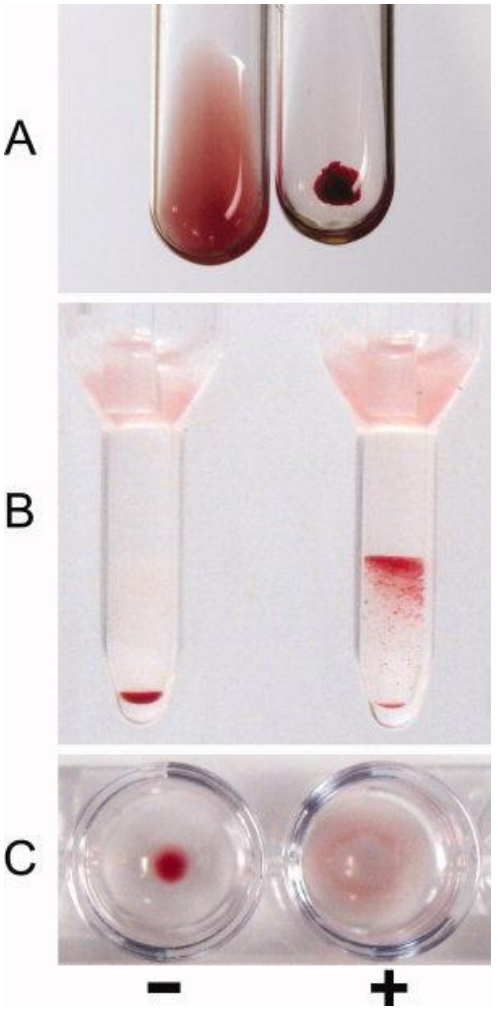


- Indirect antiglobulin test
  - Detection of allo or autoantibodies present in the patient's serum
- Direct antiglobulin test
  - Detection of allo or autoantibodies or complement bound in vivo to patient's RBC
- Polyspecific anti-human globulin reagent does not contain anti-IgA
- Repeat the DAT with anti-IgA, anti-IgG, anti-IgM, anti-C3c and anti-C3d to confirm the DAT to be negative if suspiscion of AIHA is strong

Autoimmune hemolytic anemia: etiology

- Idiopathic AIHA
- Congenital abnormalities of the immune system
  - Common variable immunodeficiency
- Viral infection
  - EBV, HIV
- Autoimmune diseases
  - LED
  - RA, scleroderma, ulcerative colitis…
- Lymphoma
  - CLL: 11%
  - NHL: 3%

The direct antiglobulin test: A critical step in the evaluation of hemolysis



visually

Fully automated laboratory systems

American Journal of Hematology
Volume 87, Issue 7, pages 707-709, 6 MAY 2012 DOI: 10.1002/ajh.23218
http://onlinelibrary.wiley.com/doi/10.1002/ajh.23218/full#fig1

The direct antiglobulin test: A critical step in the evaluation of hemolysis

TABLE I. Causes of Positive and Negative Direct Antiglobulin Test (DAT) Reactions

Positive

1. Normal
2. Autoimmune hemolytic anemia (primary and secondary causes)
   a. Warm autoimmune hemolytic anemia
   b. Cold autoimmune hemolytic anemia
   c. Mixed autoimmune hemolytic anemia
   d. Paroxysmal cold hemoglobinuria
3. Transfusion related
   a. Acute hemolytic transfusion reaction
   b. Delayed hemolytic transfusion reaction
   c. Delayed serological reaction
   d. Passive transfer of antibody by transfusion
4. Hemolytic disease of the fetus/newborn
5. Passenger lymphocyte syndrome
6. Drug-induced hemolytic anemia
7. Passive transfer of antibody in immunoglobulin preparations
   a. Intravenous immune globulin (IVIG)
   b. Rh_0(D) immune globulin
8. False positive
   a. Spontaneous red blood cell agglutination
   b. Wharton's jelly in cord blood specimens
   c. Technical
      i. Poor washing technique
      ii. Improper agitation of specimen during reaction strength determination (conventional test tube method)
      iii. Over-centrifugation
      iv. Clotted specimens

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The direct antiglobulin test: A critical step in the evaluation of hemolysis

TABLE I. Causes of Positive and Negative Direct Antiglobulin Test (DAT) Reactions

Negative

1. Nonimmune causes of hemolysis
2. Drug-induced hemolytic anemia
3. Hemolysis due to an IgA or IgM immunoglobulin
4. Low level of bound antibody and/or complement
5. Low affinity antibody
6. False negative
   a. Poor washing technique
   b. Improper agitation of specimen during reaction strength determination (conventional test tube method)
   c. Failure to add or delayed addition of antihuman globulin (AHG) reagent
   d. Inactive antihuman globulin (AHG) reagent
   e. Inappropriately concentrated red blood cell suspension
   f. Delay in testing

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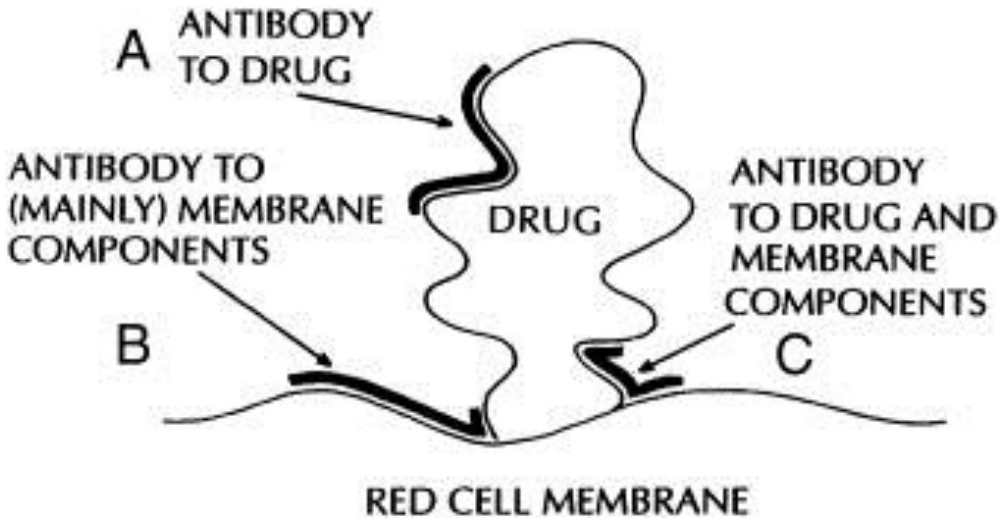
Proposed unifying hypothesis of drug-induced antibody reactions. The thicker, darker lines represent antigen-binding sites on the Fab region of the drug-induced antibody. (A) Drugs (haptens) bind loosely (or firmly) to cell membranes, and antibodies...



George Garratty

Immune hemolytic anemia associated with drug therapy

Blood Reviews Volume 24, Issues 4–5 2010 143 - 150

http://dx.doi.org/10.1016/j.blre.2010.06.004

Immune hemolytic anemia associated with drug therapy

Blood Reviews 2010;24:143

Table 1

Number of cases/fatalities of DIIHA encountered by us over a 10 year period.[a]

Drug	2000–2009 (10 years)	
	Number	Percentage
Cefotetan	36 (4)	43
Ceftriaxone	17 (5)	21
Piperacillin	14 (1)	17
β-lactamase inhibitors	6	7
Other cephalosporins	1	1
Others	9[b]	11
Total	83 (10)	100

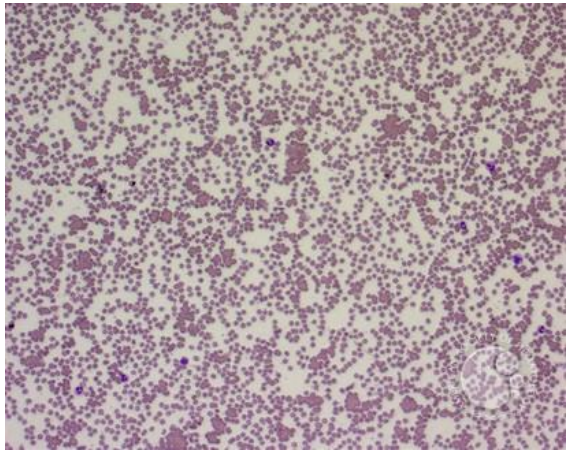
[a] Columns contain number (fatalities) of cases associated with each drug.

[b] Oxaliplatin (3), carboplatin (1), rifampin (1), diclofenac (1), cimetidine (1), sulfamethoxazole (1), and trimethoprime (1).

Autoimmune hemolytic anemia

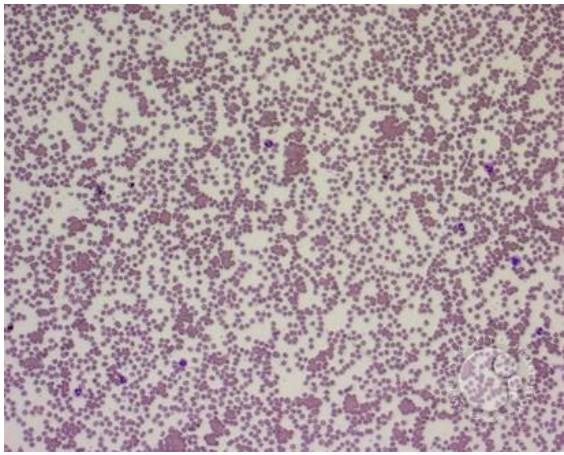
- Cold antibody AIHAs
  - Cold agglutinin disease
  - Paroxysmal cold hemoglobinuria

Autoimmune hemolytic anemia:Cold agglutinins disease



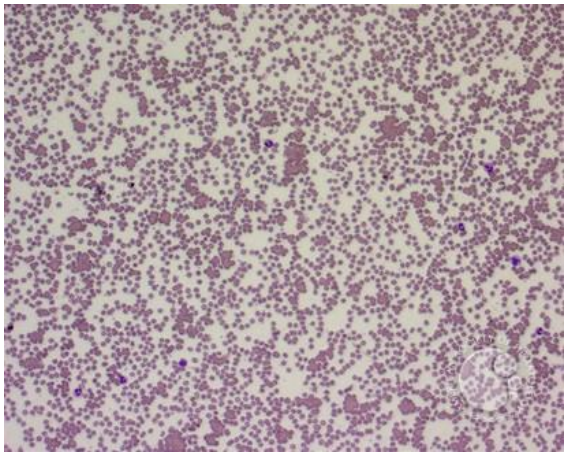
- 15% of AIHA cases
- 1 per million people per year
- IgM-mediated process in 90% of patients
- Finding of agglutination without antiglobulin antisera in microtiter wells at 4° C
- Extravascular and intravascular hemolysis mediated by complement

Autoimmune hemolytic anemia:Cold agglutinins disease



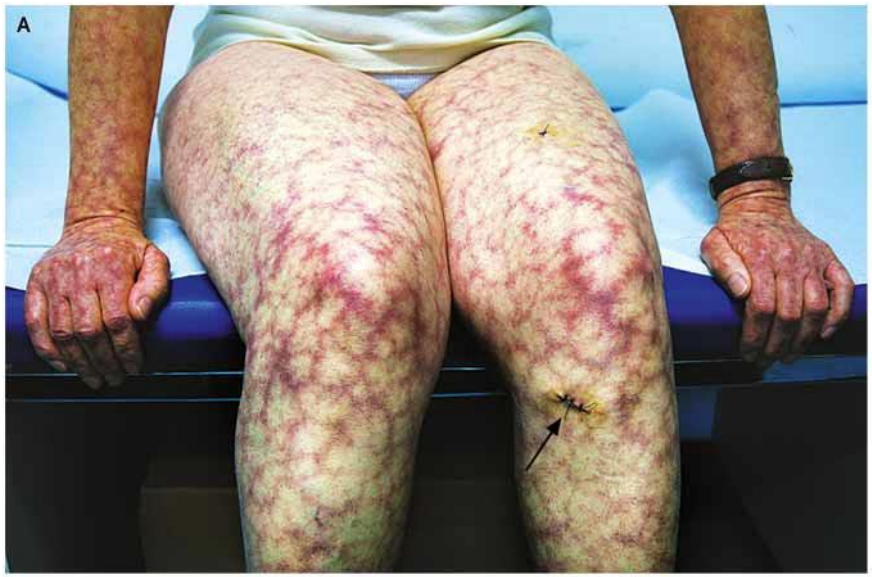
- Polyclonal or monoclonal
  - Polyclonal: usually children and young adults
    - post-infectious setting: Mycoplasme, EBV, Legionelle, CMV, …
      - Usually self-resolving
    - Monoclonal: usually older patients
      - Long-term disease
      - Frequent resistance to therapy
      - May be associated with lymphoproliferative disease

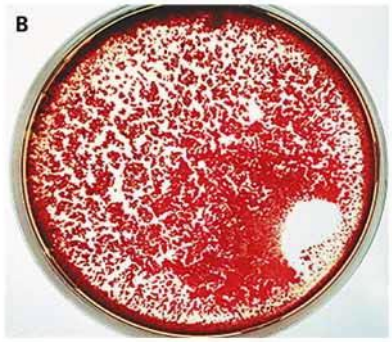
Autoimmune hemolytic anemia:Cold agglutinins disease

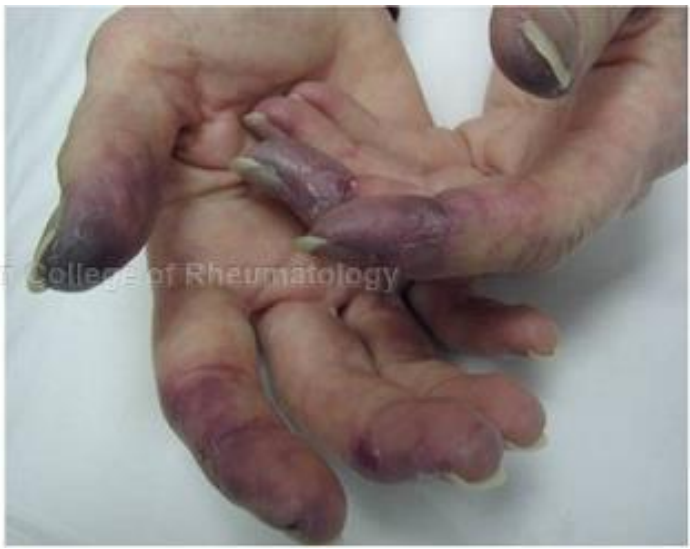


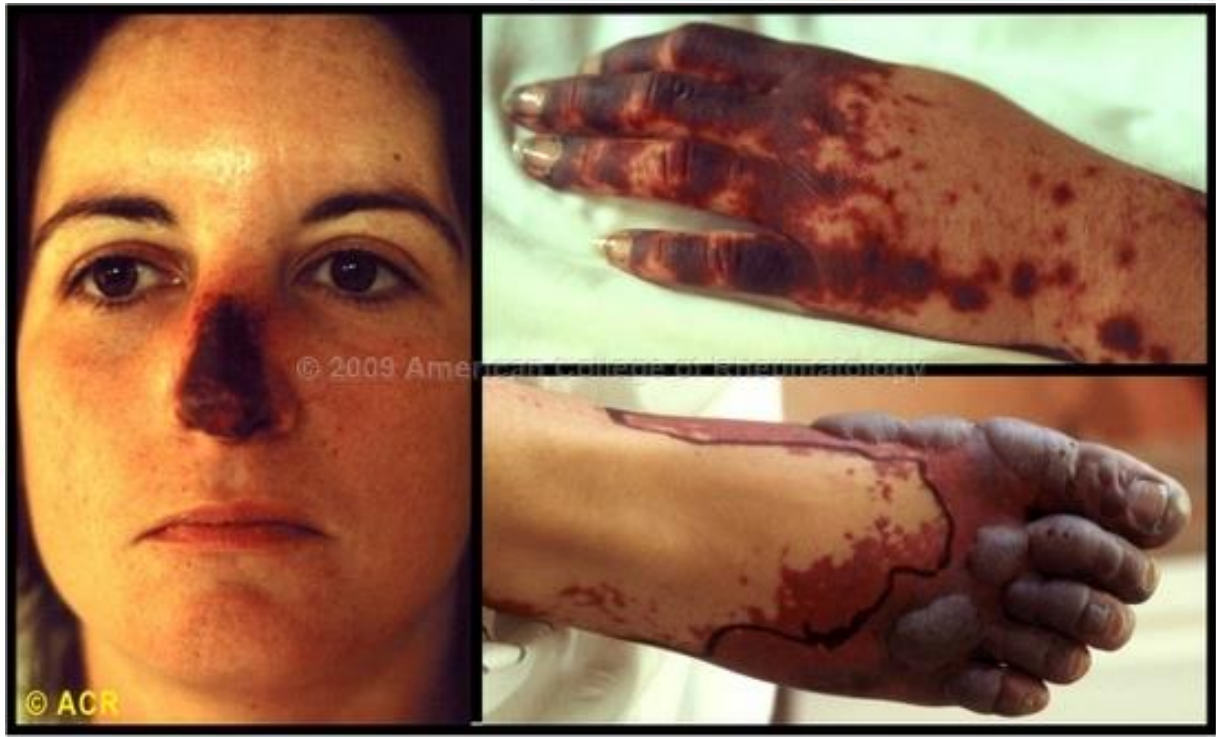
- Clinical manifestations
  - Hemolysis
    - Transfusion: > 50% of patients
    - Therapy necessary in 70% of patients
  - Cold-induced circulatory symptoms
    - Livedo reticularis
    - Raynaud disease
    - Acrocyanosis
    - Cutaneous necrosis
  - Splenomegaly: rare

Cold agglutinin disease: clinical manifestations

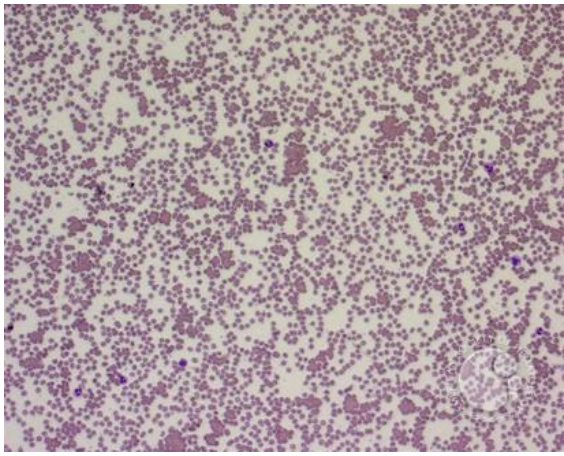








Autoimmune hemolytic anemia:Cold agglutinins disease



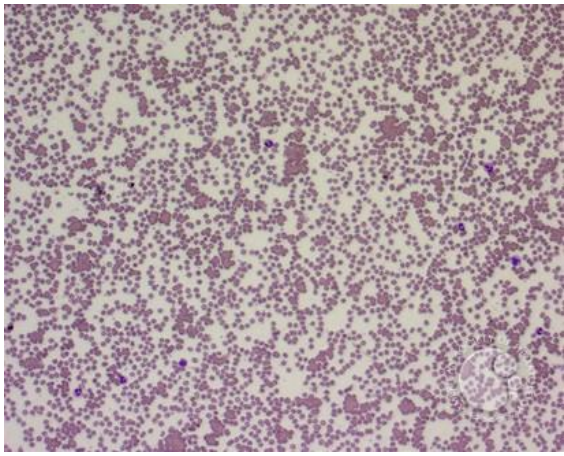
- Diagnosis
  - Hemolytic anemia
  - Reticulocytosis
  - Hyperbilirubinemia
  - Low haptoglobin
  - LDH
  - Positive Coombs testing
    - for anti-C3
    - Classically negative anti-IgG
  - Most agglutinins clinically non significant (only 14%)
  - Titer: significant when > 1/512
- After diagnosis
  - Infection? Malignancy? Autoimmune disease?

Autoimmune hemolytic anemia:Cold agglutinins disease

Table 2. Clinical features (N = 89)

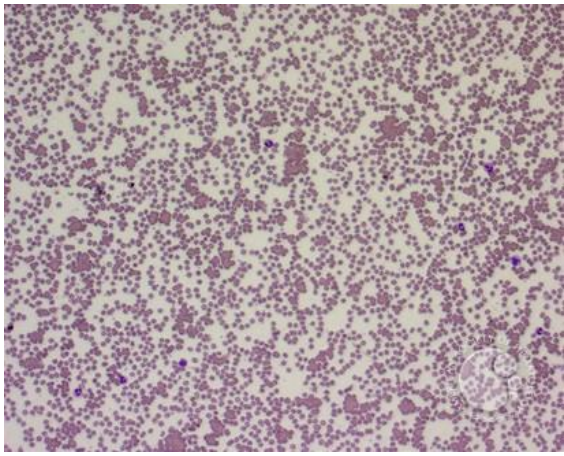
Characteristic	No.	%
Time from symptoms to diagnosis, mo		
Median	37.4	
Range	0-374.2	
Chief concern at diagnosis		
Anemia of undetermined origin	31	35
Acrocyanosis	21	24
Fatigue	19	21
Weakness	4	4
Dyspnea on exertion	3	3
Hemoglobinuria	3	3
Symptoms during disease course		
Acrocyanosis	39	44
Fatigue	36	40
Dyspnea on exertion	19	21
Hemoglobinuria	13	15
Weakness	9	10
Weight loss	9	10
Identification of triggers		
Cold	35	39
Other	20	22
Received drug therapy	73	82
Received transfusion	36	40
Overall survival, y		
Median	10.6	
Range	0.0-29.9	
Survived 5 y after diagnosis	68	76

90%,in an other report



Blood 2013;122:114-1121

Autoimmune hemolytic anemia:Cold agglutinins disease



Underlying hematologic disease in 76% of patients

Table 3. Underlying hematologic diagnoses (n = 69)

Hematologic diagnosis	No. of patients*	%
MGUS	42	61
Macroglobulinemia	6	9
Unspecified lymphoproliferative disorder	8	12
Other lymphoma†	8	12
CLL	4	6
Cutaneous T-cell lymphoma	1	1

CLL, chronic lymphocytic lymphoma.

*Disease-specific percentages were calculated by using the number of patients in the subgroup as denominators.

†Including low-grade B-cell and diffuse large B-cell lymphomas.

Blood 2013;122:114-1121

Autoimmune hemolytic anemia:Cold agglutinins disease

89 patients from 1970 through 2012

Cold avoidance!!!

Table 4. Treatment characteristics

Characteristic	Corticosteroid				Rituximab				Purine analog		Alkylating agent		Other therapy*	
	Single-agent prednisone		Any corticosteroid-containing therapy		Single-agent rituximab		Any rituximab-containing therapy							
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Treatments	26		35		47		77		10		59		24	
Patients treated	24		30		32		44		8		37		21	
Used as first-line therapy	21	81	23	69	14	30	23	30	2	20	21	36	7	29
Used as part of dual- or multi-modality therapy	—		7	20	—		30	39	6	60	39	66	14	58
Duration of therapy mo†														
Treatments with available data	19		27		38		65		5		41		14	
Median	3.0		1.9		1.0		1.0		4.0		3.2		2.8	
Range	0.1-13.9		0.1-120.0		0.1-20.4		0.1-107.7		1.0-24.5		0.0-46.4		0.0-108.7	
Tolerated therapy	22	85	29	83	42	89	71	92	9	90	49	83	21	88
Patients with known underlying hematologic disorder	18/24	75	24/30	80	31/32	97	38/44	86	7/8	88	31/37	84	17/21	81
Lymphoproliferative disorder‡	10		12		16		20		7		17		12	
Other hematologic disorder§	8		12		15		18		0		14		5	
Concurrent need for transfusion‖	9	35	12	34	6	13	16	21	2	20	14	24	9	38
Decreased need after therapy	1	11	3	25	6	100	10	63	1	50	3	21	2	8
Increased need after therapy	0	0	0	0	0	0	0	0	0	0	1	7	3	13
Confirmed response to therapy¶	9	36	14	42	39	83	61	79	5	63	26	46	11	48
Treatments with available data	25		33		47		77		8		57		23	
Duration of response, mo														
Median	60.0		51.6		27.0		24.0		18.5		11.3		36.6	
Range	2.9-210.8		7.0-210.8		2.0-135.6		1.0-135.6		12.0-41.7		0.4-146.8		1.0-166.5	
Further treatment required#	17	65	24	69	23	49	42	55	6	60	40	68	14	58

*Other therapy included azathioprine, erythropoietin, danazol, interferon alpha, plasma exchange, intravenous immunoglobulin, methotrexate, cyclosporine, or vincristine.
†Patients with "0" duration received 1 infusion or treatment (1 day) without continuation of therapy.
‡Includes chronic lymphocytic leukemia, macroglobulinemia, unspecified lymphoproliferative disorders, and other lymphomas. T-cell lymphoma was excluded.
§Includes MGUS and T-cell lymphoma.
‖Transfusion requirement was noted for each individual round of therapy, not overall patient course, to reflect the effect of the unique therapeutic agent.
¶Response to therapy was noted for each round of therapy, not overall patient treatment course, to reflect the unique response to the specific therapeutic regimen.
#Requirement for further treatment was noted for each round of therapy to reflect their individual efficacies.

Rituximab associated with high response rates but complete and sustained remissions are rare

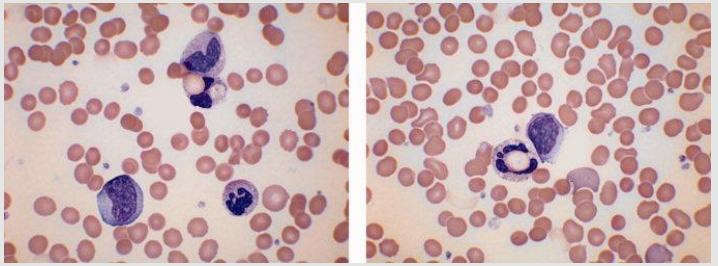
Blood 2013;122:114-1121

Paroxysmal cold hemoglobinuria

Petz: cold antibody autoimmune hemolytic anemias. Blood Reviews 2008;22:1-15

Table 4 Paroxysmal Cold Hemoglobinuria Characteristic Clinical Manifestations.

- Symptoms and Signs
  - Patient Usually a Child
  - History of a recent upper respiratory or ''flu-like'' illness.
  - Acute onset of Illness
    - Fever
    - Malaise
    - Abdominal Pain
  - Red or Red-Brown Urine is Frequently Present
  - Jaundice
  - Pallor
- Laboratory Findings
  - Anemia
    - Often Severe
    - May be Rapidly Progressive
  - Reticulocytosis (Reticulocytopenia in Some Patients)
  - Abnormal RBC Morphology
    - Spherocytosis, anisocytosis, poikilocytosis, autoagglutination, Polychromatophilia.
    - Erythrophagocytosis by neutrophils is commonly present
    - Hemoglobinuria
  - Erythroid Hyperplasia or Normal Results in Bone Marrow
  - Leukocytosis (Leukopenia in Some Patients)
  - Platelet Count usually Normal or Elevated



Paroxysmal cold hemoglobinuria

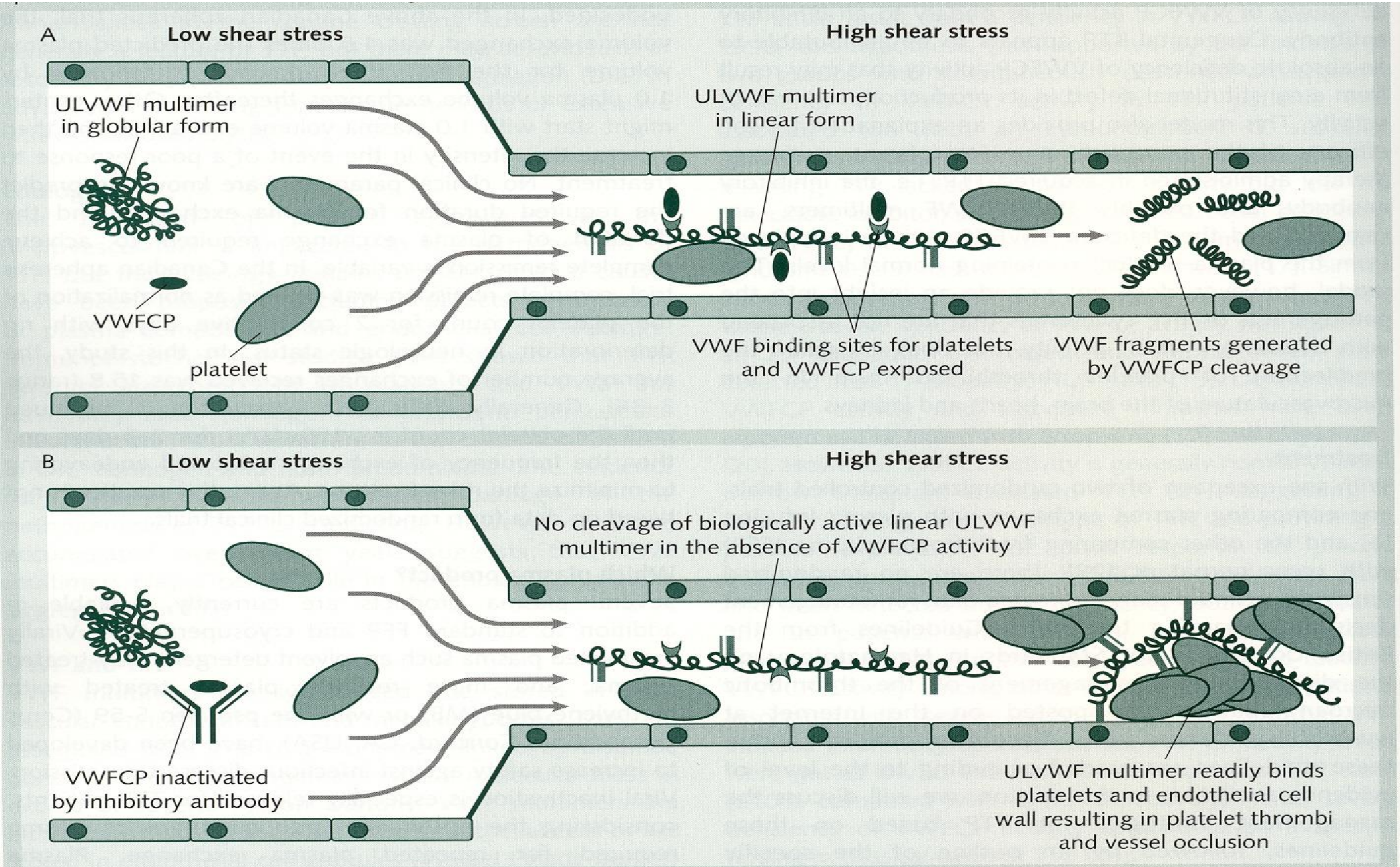
Petz: cold antibody autoimmune hemolytic anemias. Blood Reviews 2008;22:1-15

Table 5 Comparison of typical characteristics of the antibody in cold agglutinin syndrome with the Donath-Landsteiner antibody of paroxysmal cold hemoglobinuria.

	Cold agglutinin Syndrome	Donath-Landsteiner Antibody
Titer (4 °C)	high (>500)	moderate (<64)
Thermal range	high (>30 °C)	moderate (< 20 °C)
Bithermic lysis (Donath-Landsteiner test)	negative	positive
Immunoglobulin class	IgM	IgG
Specificity	anti-I or i	anti-P

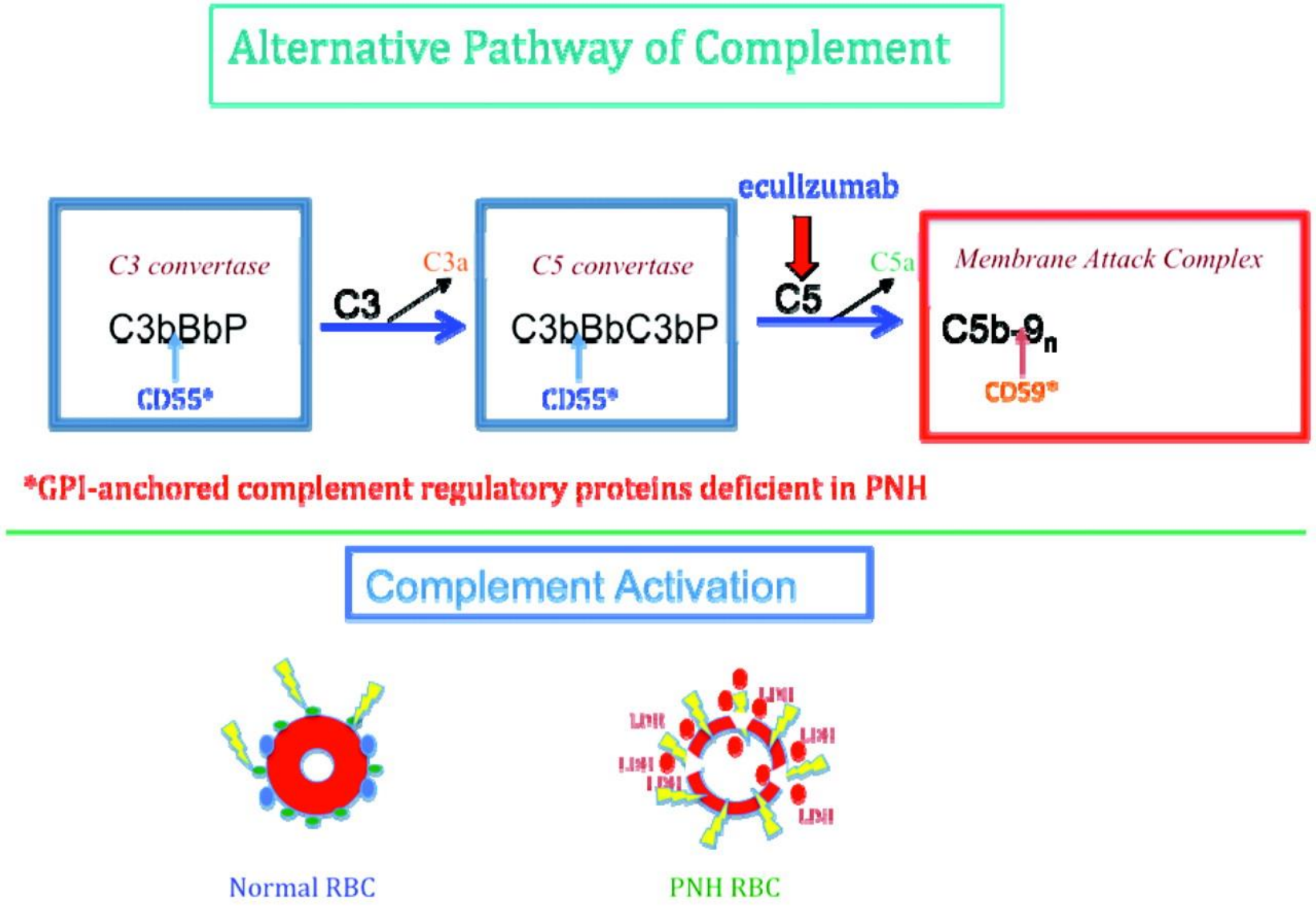
Biphasic hemolysin
Sensitizes RBCs in the cold
Induces hemolysis when the RBCs reach 37° C
Normal RBC incubated with the patient's serum at 0° C and moved ato 37° C for further incubation
No lysis following incubation at 0° C
No lysis if incubation at only 37° C

TTP



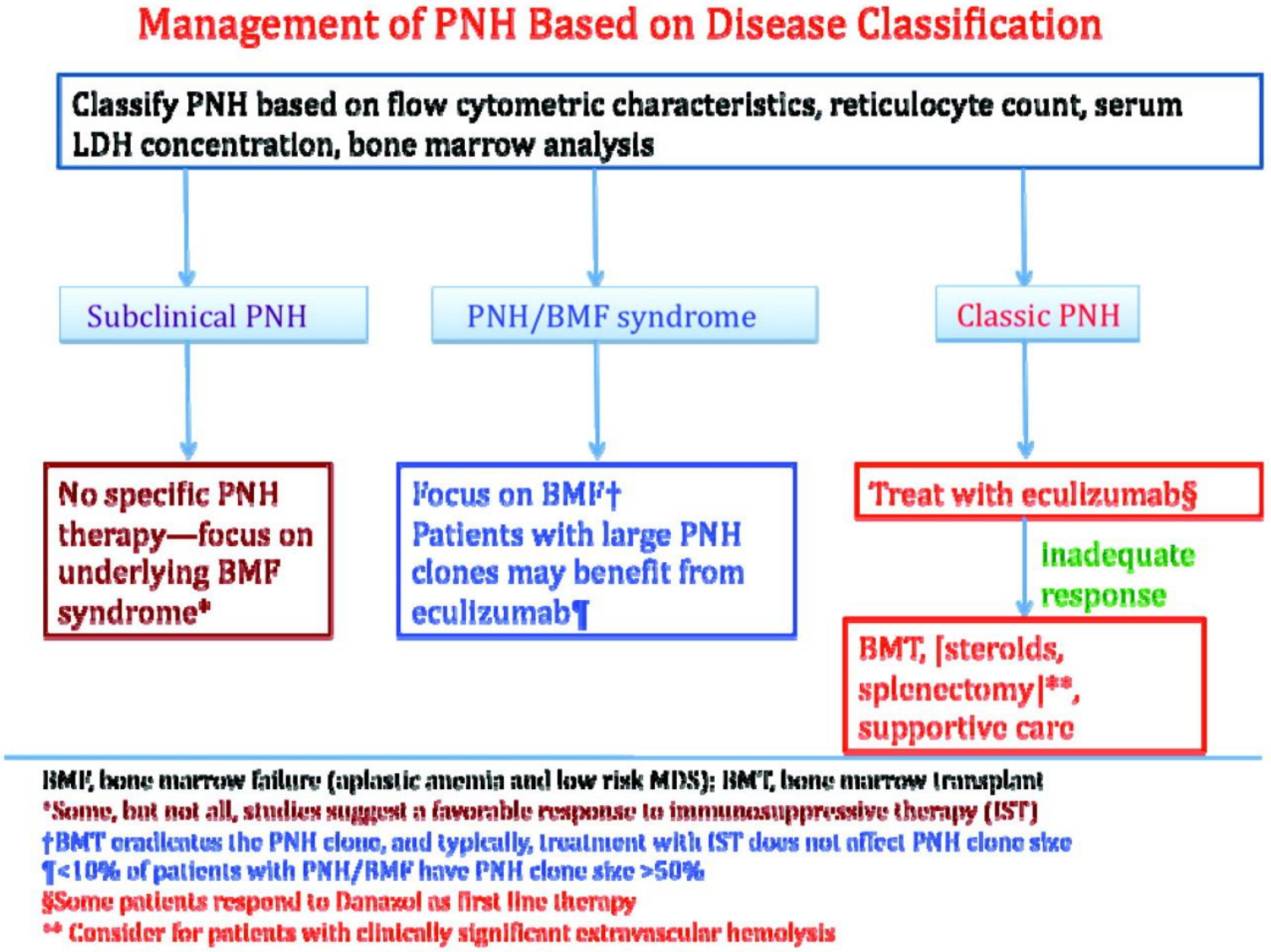
TTP: thrombotic thrombocytopenic purpura; ULVWF: ultra-large von Willebrand factor; VWFCP: von Willebrand factor cleaving protease.

Complement-mediated lysis of PNH erythrocytes.



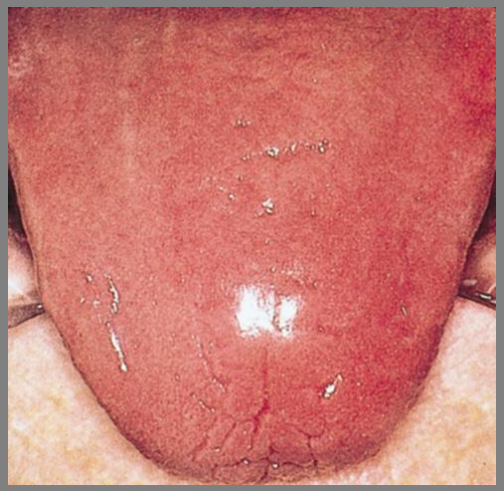
Parker C J Hematology 2011;2011:21-29

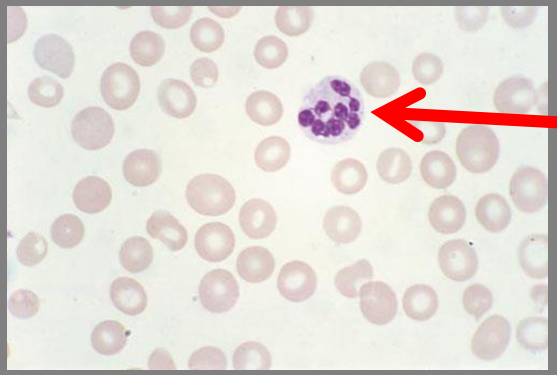
Treatment algorithm based on disease classification.

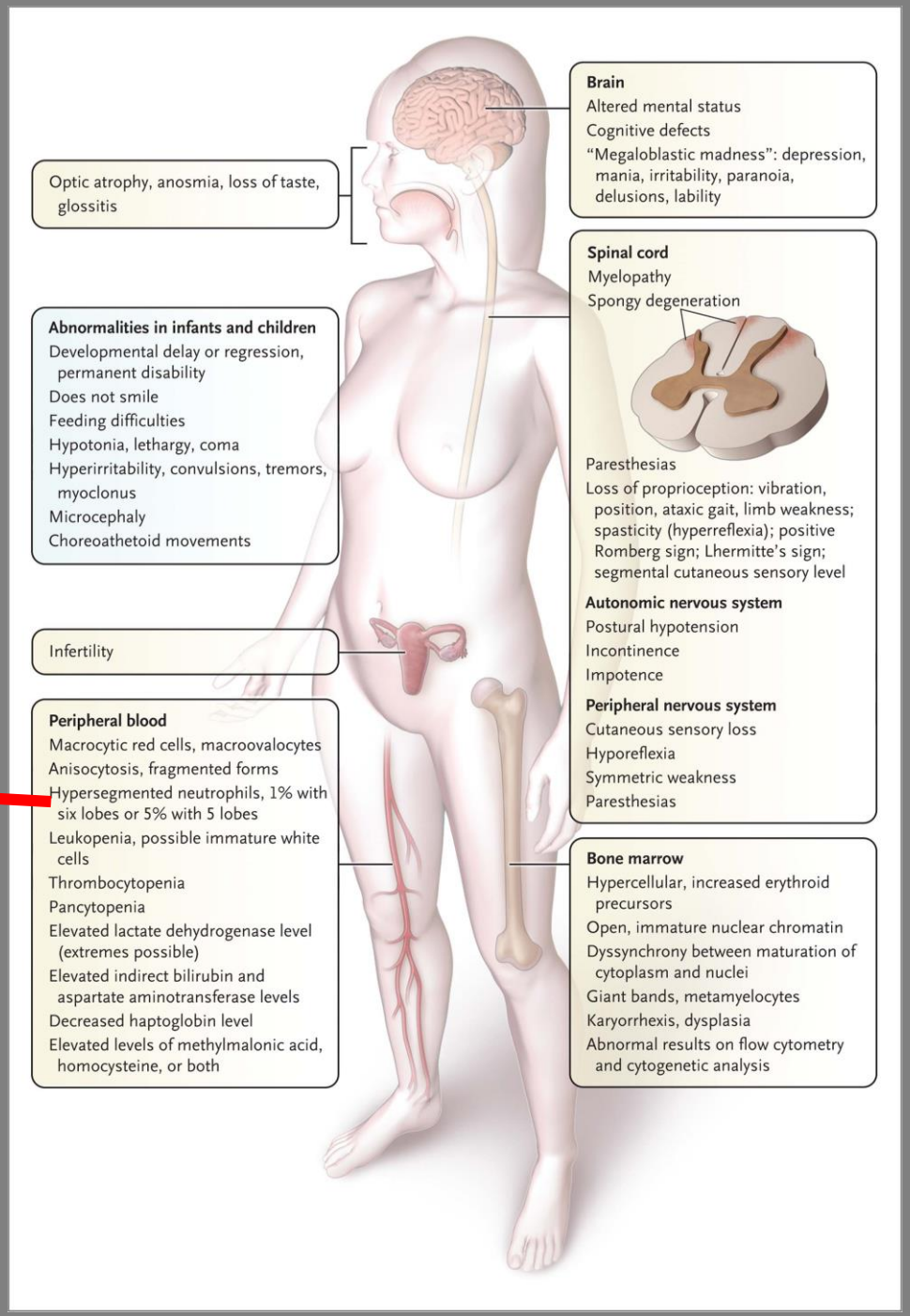


Parker C J Hematology 2011;2011:21-29

Clinical and Laboratory Findings in Vitamin B_{12} Deficiency.



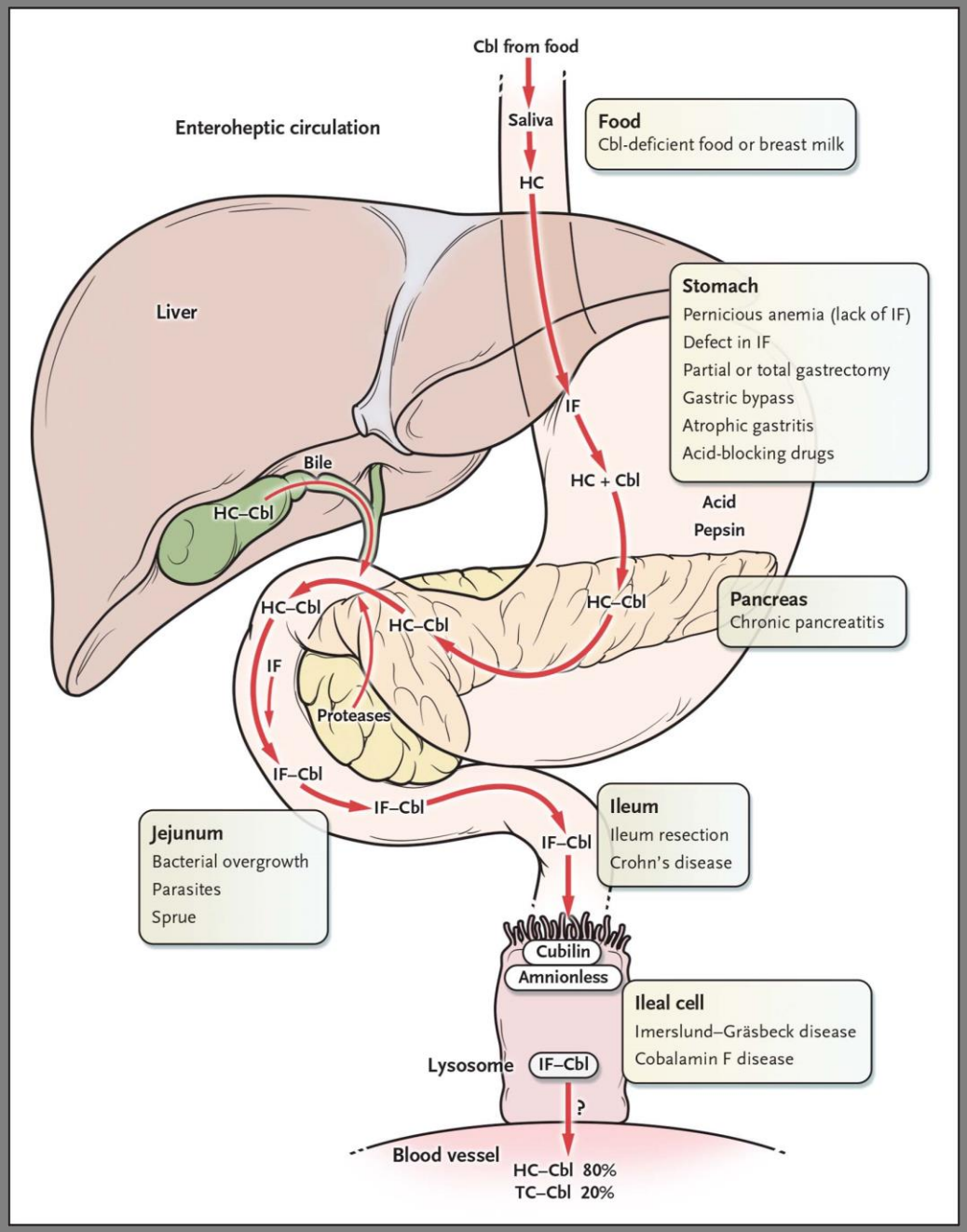




Stabler SP. N Engl J Med 2013;368:149-160

The NEW ENGLAND JOURNAL of MEDICINE

The Normal Mechanisms and Defects of Absorption of Vitamin B_{12}.



HC: haptocorrin
TC: transcobolamin

Stabler SP. N Engl J Med 2013;368:149-160

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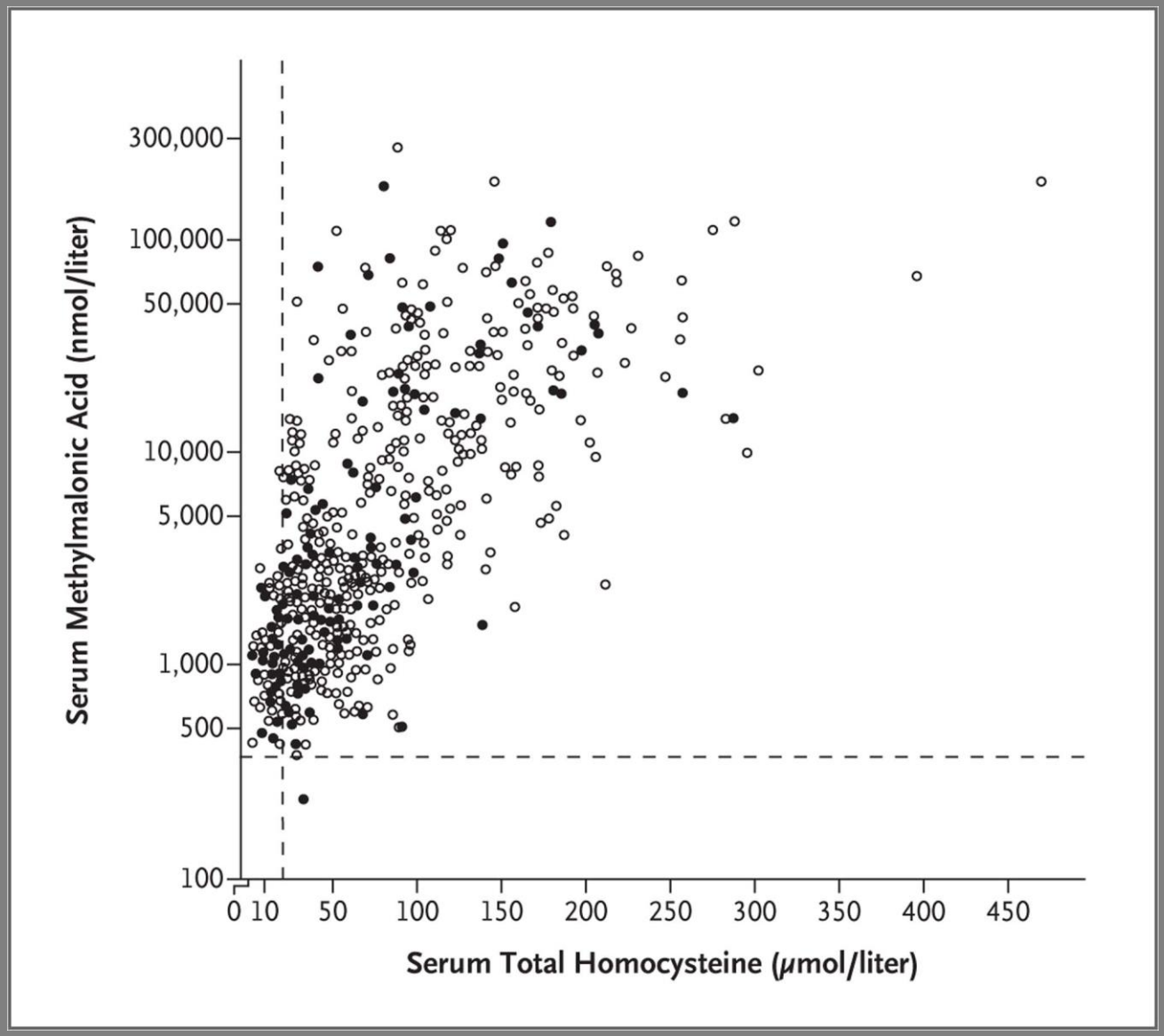
Serum Methylmalonic Acid and Total Homocysteine Concentrations in 491 Episodes of Vitamin B_{12} Deficiency.



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Causes and Treatment of Vitamin B_{12} Deficiency.

Table 1. Causes and Treatment of Vitamin B_{12} Deficiency.

Cause	Treatment	Follow-up
Severe malabsorption		
Pernicious anemia (autoimmune gastritis)	Intramuscular cyanocobalamin at a dose of 1000 μg administered intramuscularly daily or every other day for 1 wk, then weekly for 4 to 8 wk, and then monthly for life, or oral cyanocobalamin at a daily dose of 1000 to 2000 μg for life*	Administer iron and folate replacement as needed for full hemoglobin response, especially in patients with intestinal disease; perform surveillance for other autoimmune conditions, especially thyroid disease in patients with pernicious anemia; perform upper endoscopy in patients with symptoms of gastric cancer† or iron deficiency
Total or partial gastrectomy	Same as for pernicious anemia	Same as for pernicious anemia
Gastric bypass or other bariatric surgery	Same as for pernicious anemia	Same as for pernicious anemia
Ileal resection or organ reconstructive surgery (ileal conduit diversion and ileocystoplasty)	Same as for pernicious anemia	Same as for pernicious anemia
Inflammatory bowel disease, tropical sprue	Same as for pernicious anemia	Same as for pernicious anemia
Imerslund–Gräsbeck and other syndromes‡	Same as for pernicious anemia	Genetic counseling to detect vitamin B_{12} deficiency in family members
Mild malabsorption		
Protein-bound vitamin B_{12} malabsorption	Oral cyanocobalamin at a dose of 500 to 1000 μg daily or intramuscular cyanocobalamin at a dose of 1000 μg daily or every other day for 1 wk, then weekly for 4 to 8 wk, and then monthly for life	Perform tests for iron deficiency, anemia of chronic kidney disease, and anemia of chronic inflammation; these conditions coexist frequently in older adults, may limit the response to treatment, and may require further treatment
Mild atrophic gastritis	Same as for protein-bound vitamin B_{12} malabsorption	Same as for protein-bound vitamin B_{12} malabsorption
Use of metformin[14]	Same as for protein-bound vitamin B_{12} malabsorption	Same as for protein-bound vitamin B_{12} malabsorption
Use of drugs that block stomach acid	Same as for protein-bound vitamin B_{12} malabsorption	Same as for protein-bound vitamin B_{12} malabsorption
Dietary deficiency		
Adults		
Vegan or vegetarian diet, or diet low in meat and dairy products	Supplements containing >2 μg of vitamin B_{12} or foods fortified with vitamin B_{12}	Perform tests for iron deficiency, which is very common
Infants		
Breast-feeding in infants with vitamin B_{12}–deficient mothers[15,16]	Intramuscular cyanocobalamin at a dose of 250 to 1000 μg daily, then weekly until patient recovers; treatment of mother to enrich breast milk; oral supplementation with 1 to 2 μg of vitamin B_{12} daily or vitamin B_{12}–enriched formula or food	Confirm metabolic response in infants or refer parents to genetics specialist for evaluation; provide nutritional counseling for mothers
Children		
Diseases similar to those causing malabsorption in adults	100 μg of intramuscular vitamin B_{12} monthly or high-dose oral vitamin B_{12} daily in younger children; treatment as per adults in older children	Confirm pernicious anemia or congenital malabsorption
Recreational or occupational abuse of nitrous oxide§	Intramuscular cyanocobalamin at a dose of 1000 μg administered on the same schedule as that for pernicious anemia above and for life if underlying pernicious anemia is present	Evaluate for vitamin B_{12} malabsorption; provide addiction counseling
Nitrous oxide anesthesia in occult pernicious anemia[17]		

* Intramuscular hydroxocobalamin can be substituted for intramuscular cyanocobalamin, but document the long-term response if it is administered at 3-month intervals.
† Experts are not in agreement about the necessity or frequency of routine upper endoscopy in patients with pernicious anemia. However, symptoms suggestive of gastric carcinoma, unexplained iron deficiency, and proven gastrointestinal blood loss should prompt a full investigation.
‡ Congenital malabsorption of vitamin B_{12} results from mutations of the ileal cubam receptor, cubilin, or amnionless (as in the Imerslund–Gräsbeck syndrome) and from mutations in gastric intrinsic factor. These syndromes are usually manifested in infancy and early childhood, although studies have shown a delay in onset even into adolescence.[18]
§ Nitrous oxide inactivates the vitamin B_{12}–dependent enzyme methionine synthase and causes formation of vitamin B_{12} analogues and gradual tissue depletion of vitamin B_{12}.

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Vitamin B12 deficiency: diagnosis

- > 300 pg/ml or > 221 pmol/l:
  - cobolamin deficiency unlikely (probability 1-5%)
- 200 to 300 pg/ml or 148 to 221 pmol/l:
  - Borderline result; cobolamin deficiency possible
- < 200 pg/ml or <148 pmol/l
  - Low; consistent with cobolamin deficiency (specificity 95 to 100%)

- When
  - Serum cobolamin value at the lower end of the normal range or in the borderline range and high degree of suspiscion (unexplained neurological complaints or macrocytosis)
  - Dosage methylmalonic acid and homocysteine

Laboratory Testing in Vitamin B_{12} Deficiency.

Table 2. Laboratory Testing in Vitamin B_{12} Deficiency.*

Test	Sensitivity	Specificity	Comments
Measurement to detect deficiency			
Serum vitamin B_{12} <200 pg/ml or laboratory cutoff level	65–95% for proven clinical deficiency†; 50% for detecting elevated level of methylmalonic acid	50–60% for clinical response†; 80% for detecting elevated level of methylmalonic acid	Current vitamin B_{12} assays are especially problematic in patients with anti–intrinsic factor antibodies
Serum vitamin B_{12} <350 pg/ml	90%	25% for detecting elevated level of methylmalonic acid	
Holotranscobalamin <20 to 45 pmol/liter‡	Insufficient data on sensitivity for clinical deficiency; 46–89% for detecting elevated level of methylmalonic acid	Insufficient data on specificity for clinical deficiency; 28–96% for detecting elevated level of methylmalonic acid	Levels of holotranscobalamin increase in renal failure; superior to measurement of total vitamin B_{12} in pregnancy, when the total level decreases
Serum methylmalonic acid >400 nmol/liter§	98% for clinical deficiency	Poor specificity for clinical response in patients with modest elevation of level of methylmalonic acid (300–1000 nmol/liter)¶	Renal failure and volume depletion may increase level of serum methylmalonic acid, but rarely to >1000 nmol/liter
Serum or plasma total homocysteine >21 μmol/liter	96% for clinical deficiency	Homocysteine level also increased in clinical folate deficiency and renal insufficiency	
Test to determine cause of deficiency‖			
Pernicious anemia			
Anti–intrinsic factor antibodies	50%	100%	Must be tested >7 days after vitamin B_{12} injection to prevent false positive result
Anti–parietal-cell antibodies	80%	50–100%	
Atrophic body gastritis (antral sparing)**			
Fasting high serum gastrin level (>100 pmol/liter)	85%		
Low level of serum pepsinogen I (<30 μg/liter)	90%		
Endoscopy with pentagastrin-fast hypochlorhydria		100%	Rarely performed
Malabsorption of vitamin B_{12}††			
Vitamin B_{12} absorption test			Schilling test no longer available
Increase in serum holotranscobalamin level after oral loading	Unknown	Unknown	Promising preclinical data, but still experimental

* To convert the values for vitamin B_{12} to picomoles per liter, multiply by 0.7378.
† Available assays are largely chemiluminescent microparticle immunoassays performed with the use of automated analyzers that in general show higher values than the radiodilution and microbiologic assays used in past studies of clinically confirmed deficiency.[4,22,24,26] Thus, these tests are likely to have lower sensitivities and specificities than the older assays.
‡ The holotranscobalamin assay has been studied widely in Europe[27-30] but is not yet commercially available in the United States. The appropriate lower end of the reference range is still under debate.[33] The values for sensitivity and specificity are reviewed in Heil et al.[29]
§ Urinary methylmalonic acid has not been extensively studied, but values greater than 2.5 μmol per millimole of creatinine suggest deficiency.
¶ Elevated levels of methylmalonic acid fall with vitamin B_{12} therapy, but an associated clinical response is highly variable, depending largely on the presence of vitamin B_{12}–related disease.
‖ Evidence of a causal pathologic process does not confirm coexisting B_{12} deficiency, since underlying gastrointestinal disease may predate the deficiency by many years.
** The relationship between atrophic body gastritis (autoimmune gastritis) and infection with *Helicobacter pylori* is variable. Antral sparing is a type of atrophic body gastritis in which the cells in the antrum can produce high levels of gastrin.
†† There is malabsorption if clinically proven vitamin B_{12} deficiency is present in a patient who eats meat, receives multivitamin therapy, or both.

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Folic acid deficiency

- Nutritional deficiency
  - Alcoholism
  - Poor dietary intake
  - Overcooked food
  - Depression
  - Nursing homes
- Malabsorption
  - Celiac disease
  - IBD
  - Short bowel syndrome
- Drugs
  - Methotrexate
  - Trimethprim
  - Ethanol
  - Phenytoin
- Increased requirements
  - Chronic hemolysis
  - Pregnancy
  - lactation

Folic acid deficiency

- Small body stores small (5-10mg) in relation to daily requirements (200 to 400 ug)
- Megaloblastosis can occur in 4 to 5 months
- No neurological changes

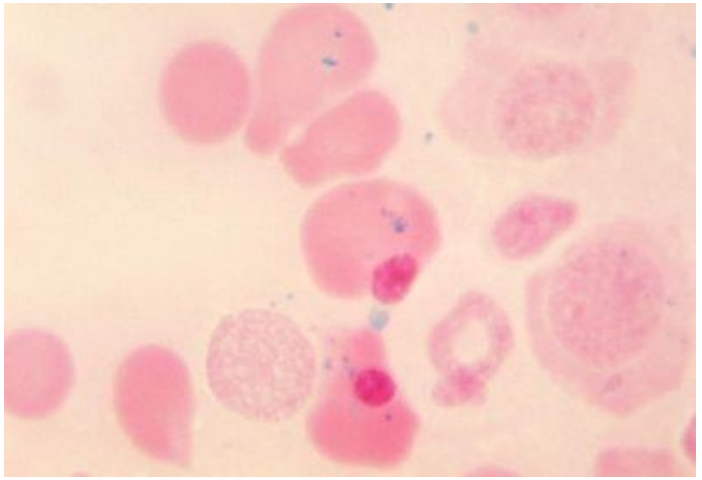
- Serum folate concentration is reflection of short term folate balance
- Red cell folate is a time-averaged value of folate availibility

- Folate > 4 ng/ml: no deficiency
- Folate < 2 ng/ml: folate deficiency
- 2 < folate < 4: borderline values: dosage of red cell folate (and homocysteine?)

- Administration of folic acid may worsen neurologic complications of untreated vit B12 deficiency

Sideroblastic anemias

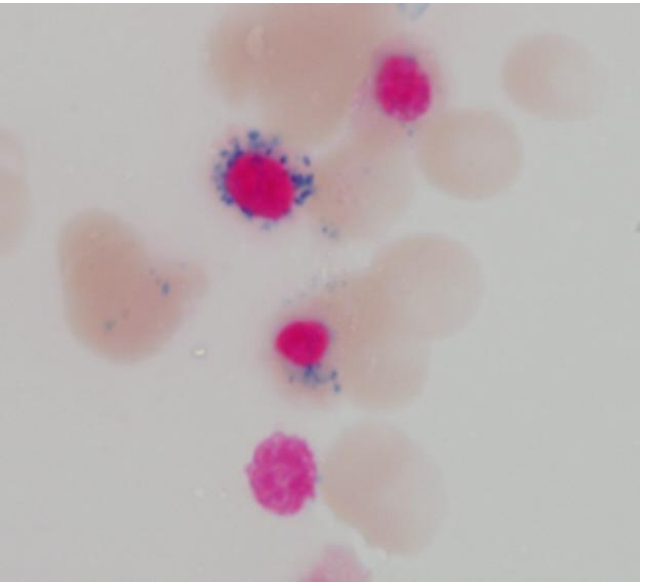
- Sideroblasts
  - Nucleated red blood cell precursors (erythroblasts) with one or more iron containing granules in the cytoplasm
  - Found in the bone marrow of normal iron-sufficient subjects
  - Normal sideroblasts show random iron deposits, typically 1-5, in the cytoplasm.

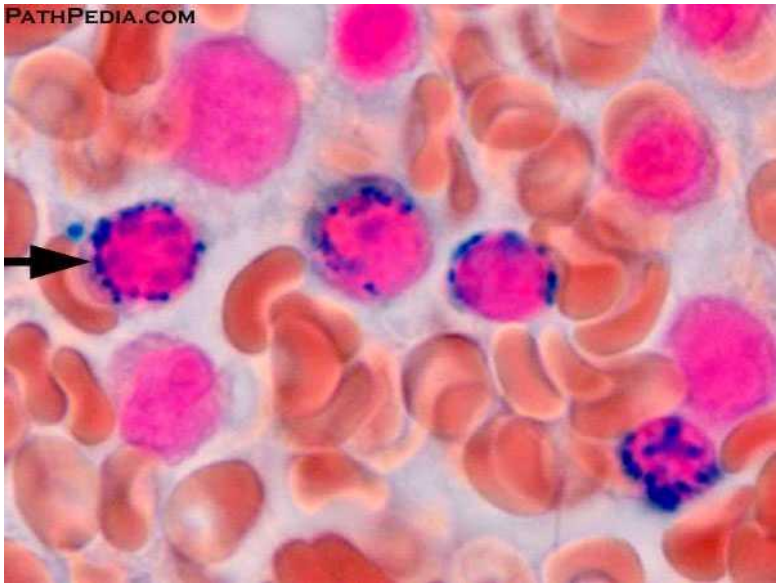


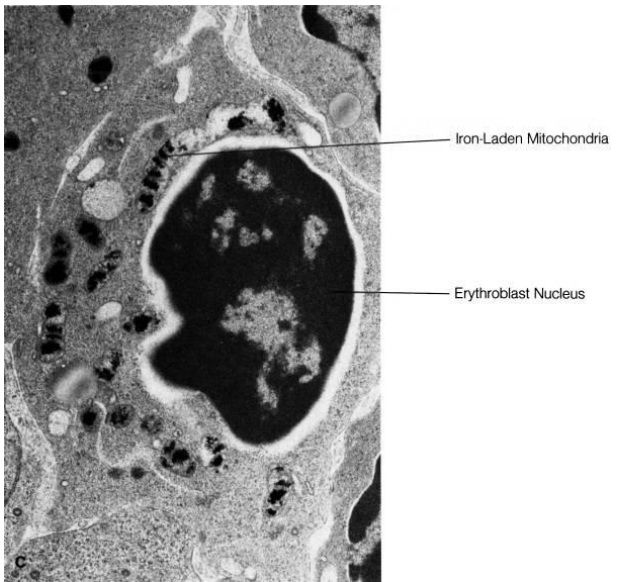
http://histology-world.com/photoalbum/displayimage.php?album=65&pid=1238#top_display_media

Sideroblastic anemias

- Abnormal sideroblasts
  - Increase of granular iron deposits around the nucleus
  - In the most abnormal form, granules completely surround the nucleus = ring sideroblasts = diagnostic feature of sideroblastic anemia
  - Reflect aberration in the processing of iron by the erythroblast
    - Insufficient production of protoporphyrin to utilize the iron delivered to erythroblasts
    - Faults in mitochondrial functions that affect iron pathways or impair mitochondrial protein synthesis





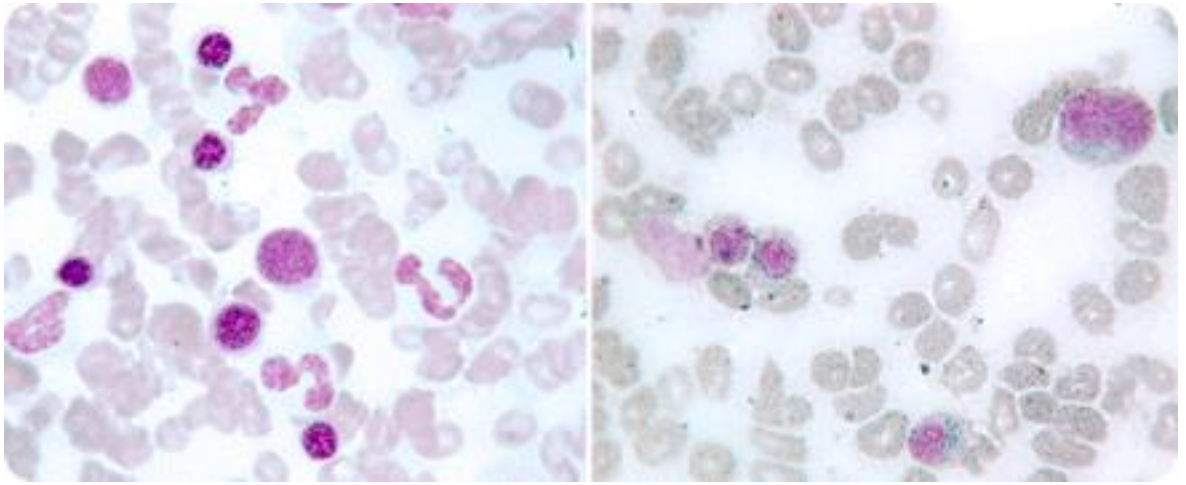


Sideroblastic anemias

- Amount of iron in BM macrophages increased due to ineffective hematopoisis

- Iron overload
  - Present in many congenital and acquired clonal forms
  - Usually absent in the acute, reversible forms

- Ring sideroblast abnormality may be masked when concomitant iron deficiency



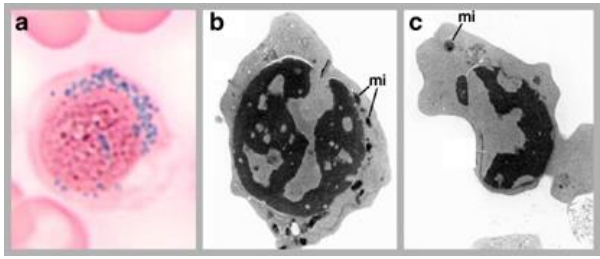
After iron repletion

Refractory anemia with ring sideroblasts masked by iron deficiency anemia Blood 2011;117:5793

Classification and causes of sideroblastic anemias

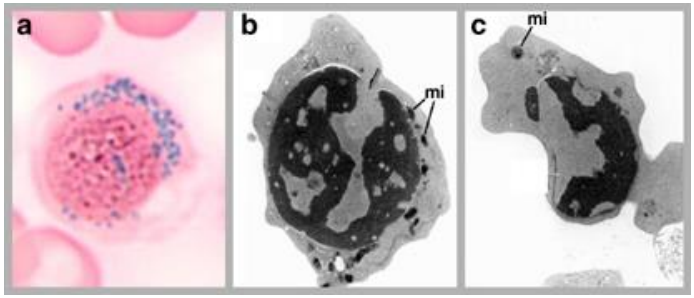
- Congenital
  - Genetically and clinically heterogeneous
  - Diverse underlying causes, inheritance patterns, clinical phenotypes and associated features
  - Syndromic affecting multiple systems
  - or non-syndromic

- Acquired
  - Clonal-Neoplastic
  - Metabolic/reversible



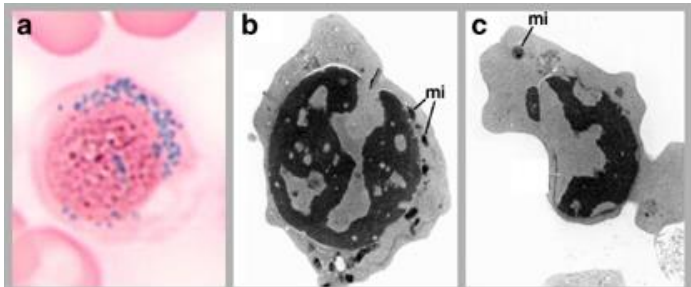
Classification and causes of sideroblastic anemias

- Acquired
  - Clonal-Neoplastic
    - Refractory anemia with ring sideroblasts (RARS)
    - Refractory anemia with ring sideroblasts and thrombocytosis (RARS-t)
    - Refractory cytopenia with multilineage dysplasia and ring sideroblast (RCMD-RS)
  - Metabolic/reversible
    - Alcoholism
    - Drugs (linezolid, isoniazid, chloramphenicol)
    - Copper deficiency
    - Hypothermia



Classification and causes of sideroblastic anemias

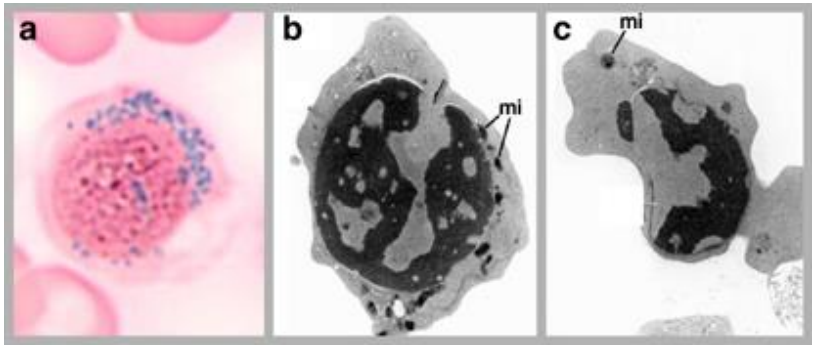
- Acquired
  - Clonal-Neoplastic
    - Refractory anemia with ring sideroblasts (RARS)
      - Pure sideroblastic anemia
      - Sometimes difficult to distinguish from X-linked sideroblastic anemia in female subjects
      - Best overall prognosis of all MDS variants
      - Pyridoxine: no response expected
    - Refractory anemia with ring sideroblasts and thrombocytosis (RARS-t)
      - Frequent presence of the JAK2 mutation
      - Mixed myeloproliferative and myelodysplastic syndrome
    - Refractory cytopenia with multilineage dysplasia and ring sideroblast (RCMD-RS)
      - True MDS



Acquired metabolic sideroblastic anemias

- Erythroid heme biosynthesis or mitochondrial functions adversely affected by acquired factors
- Sideroblatic anemia fully reversible when the offending factor is removed

  - Alcoholism
  - Drugs (linezolid, isoniazid, chloramphenicol)
  - Copper deficiency
  - Hypothermia



Different factors that can contribute to anemia and/or a low hematocrit in alcoholic patients

Table 3 Summary of the different factors that can contribute to anemia and/or a low hematocrit in alcoholic patients.

Cause of low hematocrit	Possible contributing factors
Hemorrhage and/or iron deficiency	Alcoholic gastritis Portal hypertension Peptic ulceration
Hemolysis	Chronic liver disease and/or cirrhosis Zieve syndrome Spur cell anemia of severe liver disease
Reduced erythropoiesis	Anemia of chronic disease Nutritional (e.g. folic acid deficiency) Sideroblastic anemia Alcohol toxicity
Hypersplenism	Portal hypertension
Hemodilution	Fluid retention of chronic liver disease Aggressive intravenous fluid therapy

Lewis G *et al.* (2007) A case of persistent anemia and alcohol abuse
Nat Clin Pract Gastroenterol Hepatol **4:** 521–526 doi:10.1038/ncpgasthep0922

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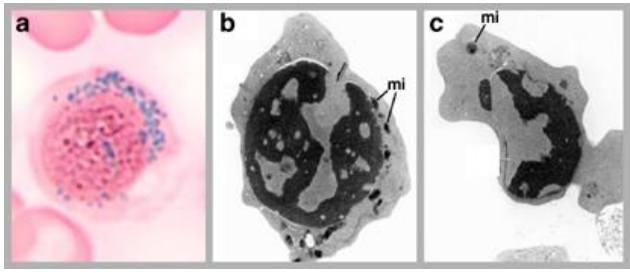
Acquired metabolic sideroblastic anemias

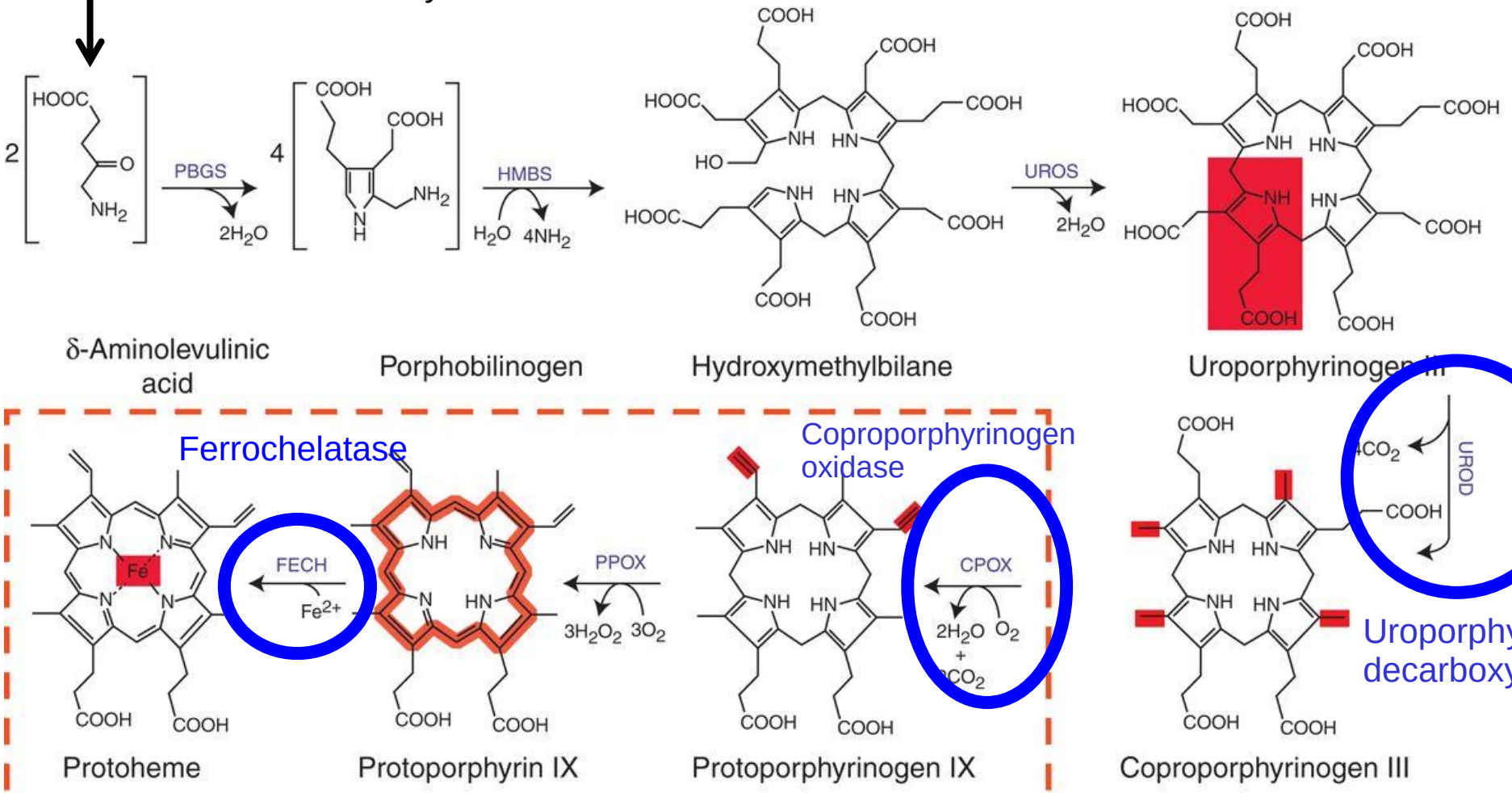


- Alcohol
  - Ring sideroblast abnormality
    - 25-30% of anemic alcoholic patients
    - Usually when malnutrition and folic acid deficiency
  - Inhibition of several enzymatic steps in the heme pathway

Succinyl-CoA + Glycine

The mammalian heme biosynthetic pathway.

5 aminolevulinate synthase



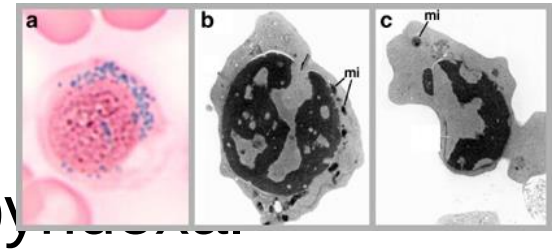
Inhibition by alcohol

Pyridoxal phosphate aids in the synthesis of hemoglobin, by serving as a coenzyme for the enzyme ALA synthase

Dailey H A , and Meissner P N Cold Spring Harb Perspect Med 2013;3:a011676

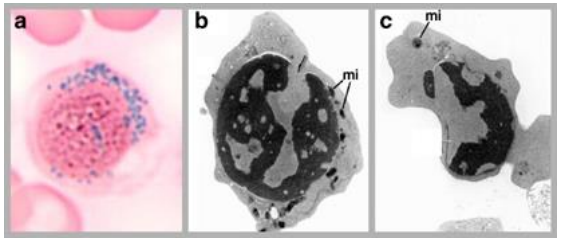
Acquired metabolic sideroblastic anemias

- Alcohol



  - Interference with vitamin B6 metabolism (pyridoxal phosphate aids in the synthesis of hemoglobin, by serving as a coenzyme for the enzyme ALA synthase)
  - Direct effect on mitochondrial protein synthesis ?
  - Withdrawal of alcohol
    - Disappearance of ring sideroblasts within days to 2 weeks
    - Resolution of anemia depends on presence or not of other problems due to alcohol

Acquired metabolic sideroblastic anemias

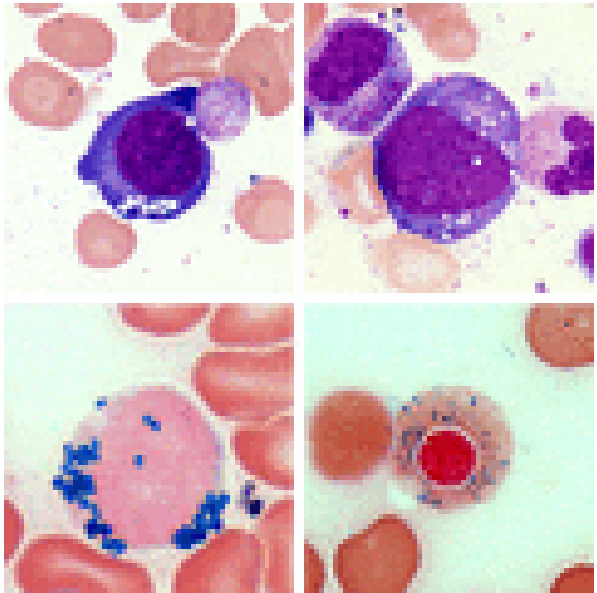


- Drugs
  - Isoniazide (INH)
    - Moderate anemia / 1 to 10 months after initiation
    - Interference with vitamin B6 metabolism
    - Reversed by pyridoxine or by withdrawal of the drug
  - Chloramphenicol
    - Causes ring sideroblast abnormality in a dose dependent manner
    - Suppresses erythropoiesis
    - Inhibition of mitochondrial protein synthesis at the level of RNA translation
  - Linezolid
    - Inhibition of mitochondrial protein synthesis

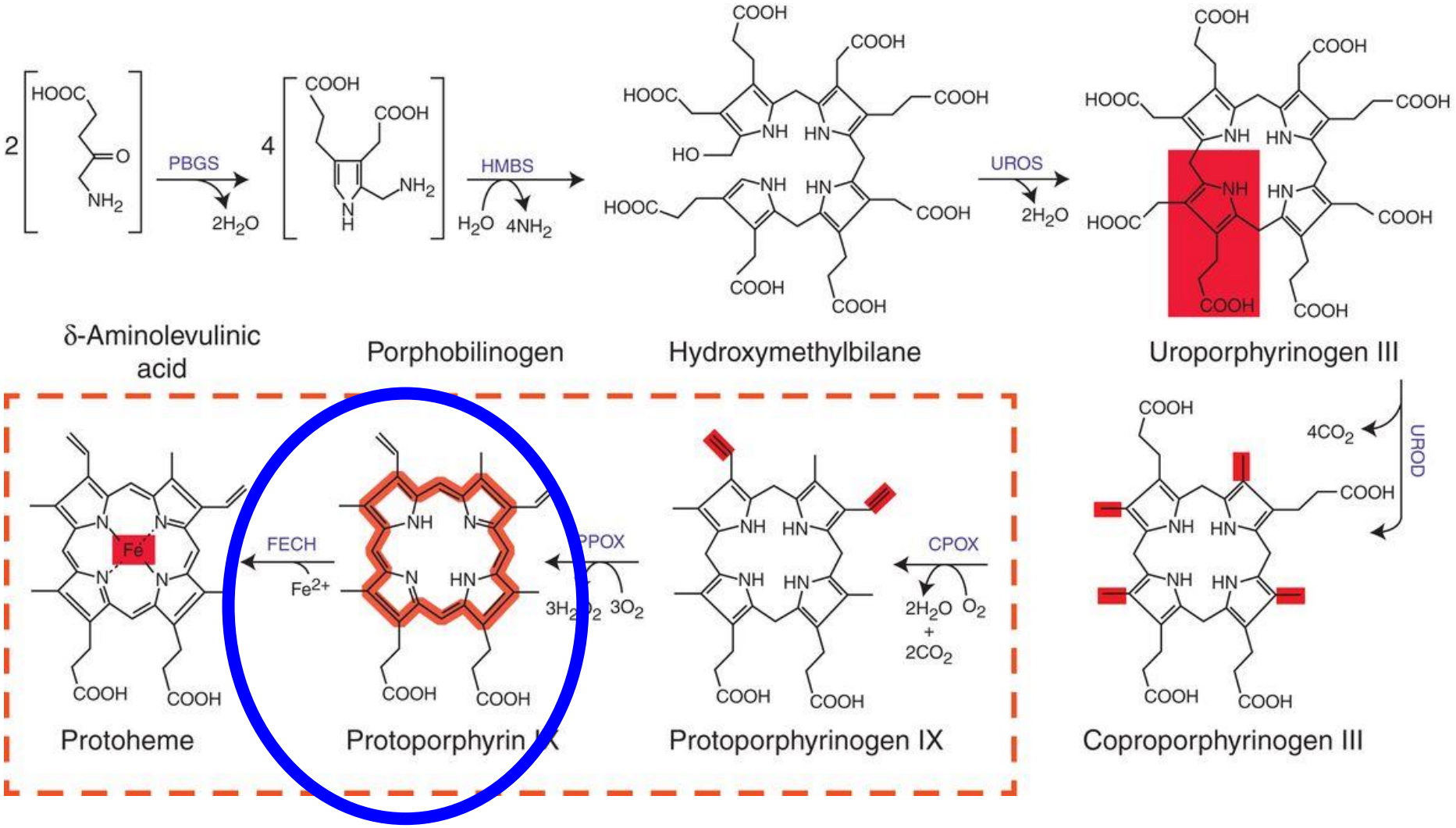
Haematologica 2013;98:e138-140

Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Impaired intestinal absorption and mobilization of iron from reticuloendothelial cells and hepatocytes because of associated lack of ceruloplasmin (ferroxidase function)
  - Decreased heme synthesis from ferric iron and protoporphyrin because of defective reduction of ferric iron to ferrous iron (diminished activity of the copper-containing cytochrome oxydase)



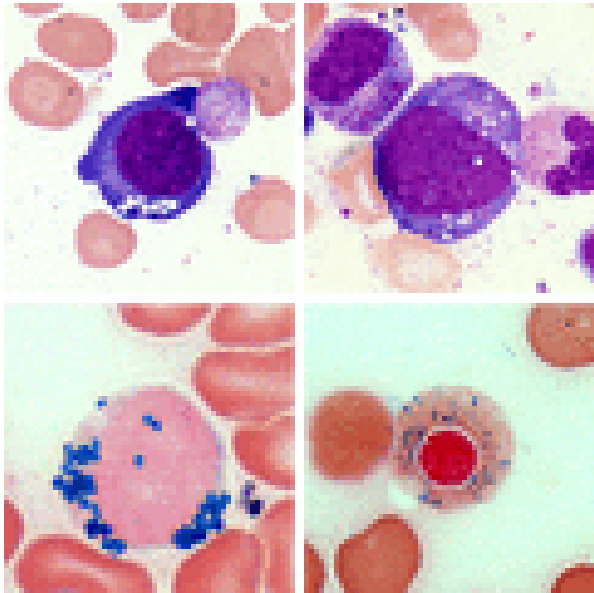
The mammalian heme biosynthetic pathway.



Dailey H A , and Meissner P N Cold Spring Harb Perspect Med 2013;3:a011676

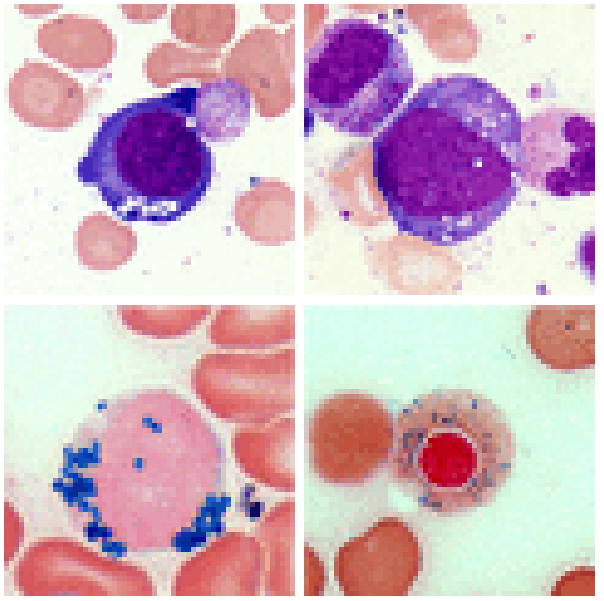
Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Impaired intestinal absorption and mobilization of iron from reticuloendothelial cells and hepatocytes because of associated lack of ceruloplasmin (ferroxidase function)
  - Decreased heme synthesis from ferric iron and protoporphyrin because of defective reduction of ferric iron to ferrous iron (diminished activity of the copper-containing cytochrome oxydase)
  - Impaired CD34+ cell differentiation and stem cell renewal



Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Prolonged parenteral nutrition
  - Prolonged enteral feeding
  - Malabsorption
  - Bariatric surgery
  - Use of large amounts of denture cream containing zinc
  - Nephrotic syndrome (urinary losses of ceruloplasmin)

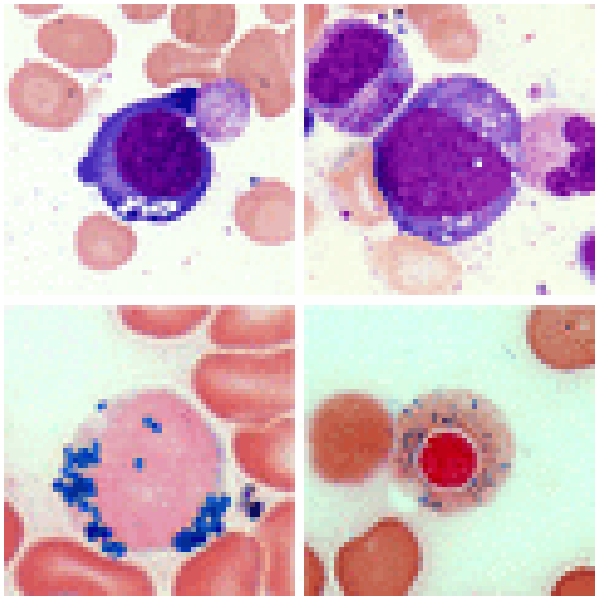


Br J Haematol 2013 Oct 14. doi: 10.1111/bjh.12577. [Epub ahead of print

Gabreyes et al. Hypocupremia associated cytopenia and myelopathy: a national retrospective review. Eur J Haematol 2013;90:1-9

Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Neurologic manifestations
    - CNS demyelination
    - Peripheral neuropathy
    - Optic neuropathy
    - Myeloneuropathy
    - Mimic subacute combined degeneration of the cord associated with B12 deficiency (! possible coexistence of the two deficiencies!)



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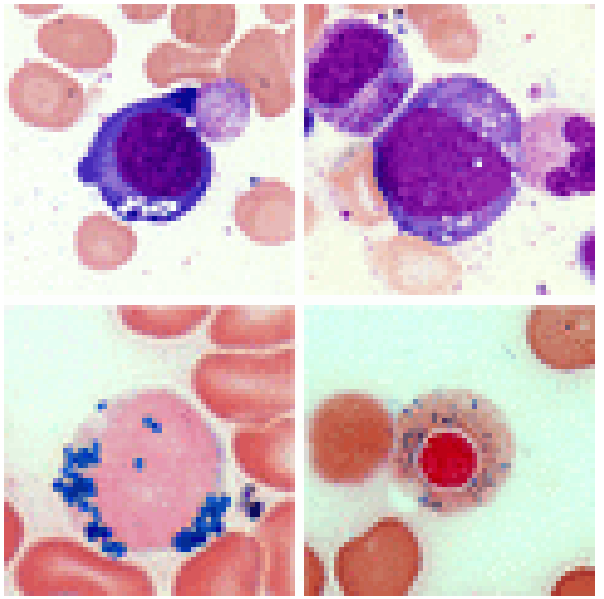
Gabreyes et al. Hypocupremia associated cytopenia and myelopathy: a national retrospective review. Eur J Haematol 2013;90:1-9

Acquired metabolic sideroblastic anemias

- Copper deficiency
  - Hematologic manifestations
    - Anemia (sometimes very severe)
    - MCV normal or slightly increased
    - Neutropenia
    - Platelet count usually normal
    - Sometimes pancytopenia
    - Serum iron and transferrin saturation normal
    - Serum copper and ceruloplasmin levels are decreased
    - Differential diagnosis: MDS (allo-BMT in one patient!!!!!)



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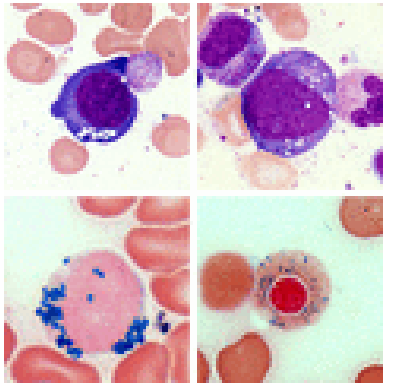
Acquired metabolic sideroblastic anemias

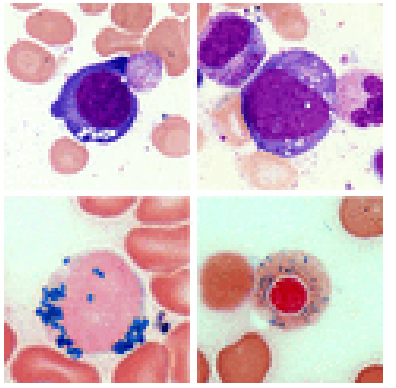
- Copper deficiency
  - Therapy:
    - parenteral or oral copper: 2 mg elemental copper/day
    - Resolution of hematologic abnormalities in < 2 months
    - Neurologic deficits may improve or only stabilize



Gabreyes et al. Hypocupremia associated cytopenia and myelopathy: a national retrospective review. Eur J Haematol 2013;90:1-9
Kumar et al: « Myelodysplasia », myeloneuropathy ans copper deficiency. Mayo Clin Proceedings 2005;80:943

Acquired metabolic sideroblastic anemias

- Hypothermia
  - Sensitivity of several mitochondrial functions to reduced temperature



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