26/04/2024

Immune mediaded Haemolytic Anemias

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slido

How do you investigate laboratory Haemolytic Anemia

ⓘ Click **Present with Slido** or install our Chrome extension to activate this poll while presenting.

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Where is the DAT test used?

ⓘ Click **Present with Slido** or install our Chrome extension to activate this poll while presenting.

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Which one of the following statements about the typical autoantibodies in warm autoimmune haemolytic anaemia is true ?

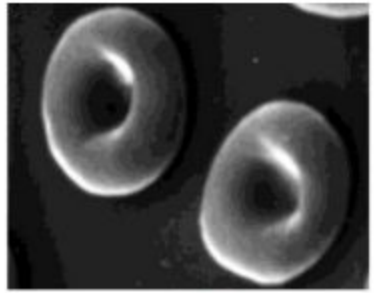
ⓘ Click **Present with Slido** or install our Chrome extension to activate this poll while presenting.

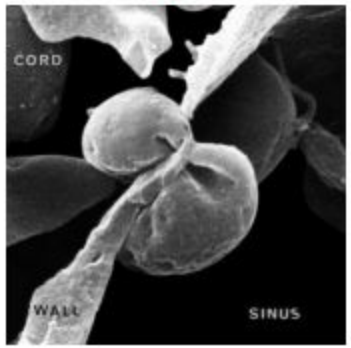
Hemolytic Anemia

- Anemia that results from the shortened survival of circulating RBC due to their premature destruction.

- The typical lifespan of a RBC is 120 days (4 months) during which time RBC are subjected to remarkable mechanical stress.
  - Highly deformable membrane and underlying cytoskeleton
    - Optimal surface to volume ratio
  - Enzymatic system that continually restores the proper redox environment of the cell.

- Hemolysis occurs when the RBC is unable to maintain this intact structure.





HEREDITARY

RBC membrane
- Hereditary Spherocytosis
- Hereditary Elliptocytosis
- Pyropoikilocytosis

RBC metabolic defects
- EM pathway
- HMP shunt
- Nucleotide Synthesis

Hemoglobin Defects
- Thalassemia
- Abnormal Variants

ACQUIRED

Immune
- Autoimmune
- Drugs

Paroxysmal Noctural Hemoglobinuria

RBC Fragmentation Syndromes

Secondary
- Renal
- Liver disease

Miscellaneous
- Toxins
- Infections
- Physical Agents

Immune mediated red cell lysis

- ❑ Warm autoimmune hemolytic anemia
- ❑ Cold agglutinin disease
- ❑ Paroxysmal cold hemoglobinuria
- ❑ Drug-induced hemolytic anemia
- ❑ Paroxysmal nocturnal hemoglobinuria

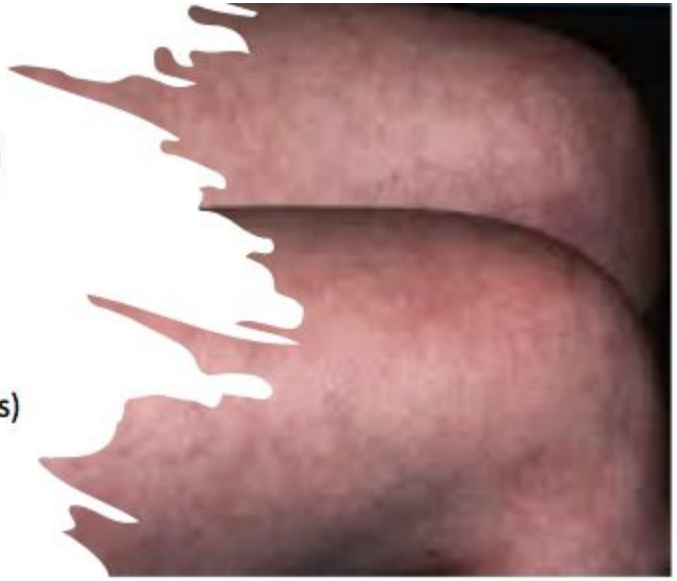
Diagnostic Workup

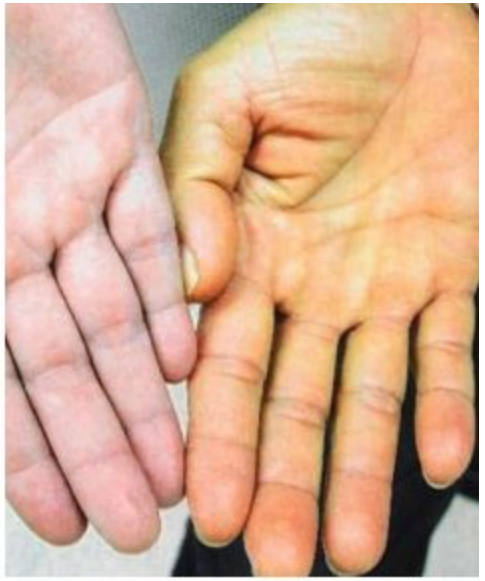
History

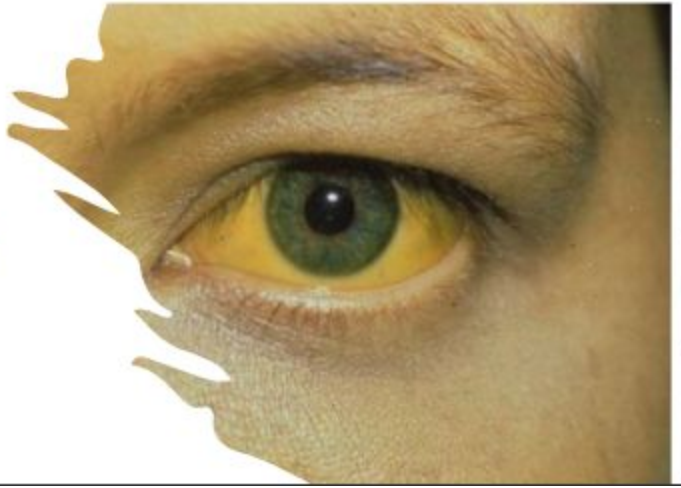
- Family history of anemia (congenital)
- Rapid onset of anemia: brisk hemolysis
- Pigmented gallstones (chronic hemolysis)
- Recent blood transfusion (acute hemolytic transfusion reaction)
- New medication (drug-induced

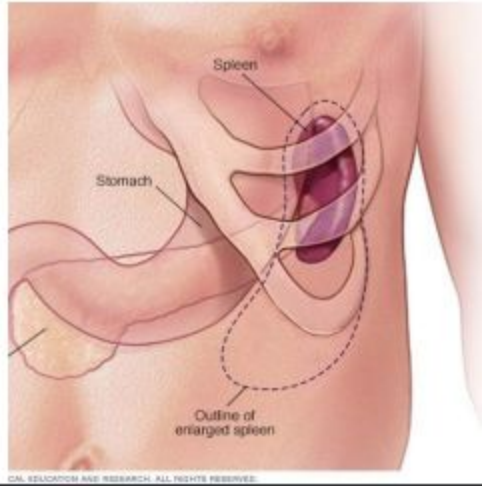
Physical Exam

- Scleral icterus
- Jaundice
- Lymphadenopathy
- Enlarged spleen (expansion of reticulo-endothelial system)
- Dark Urine



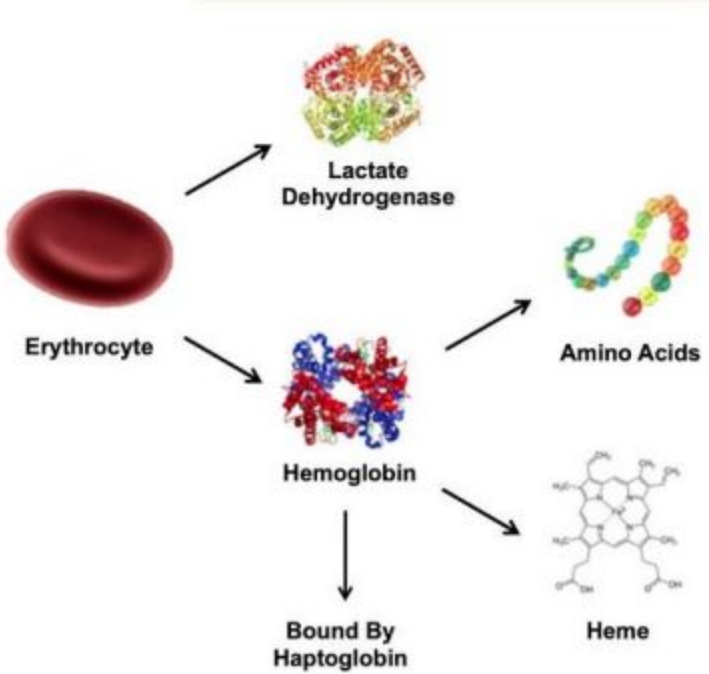




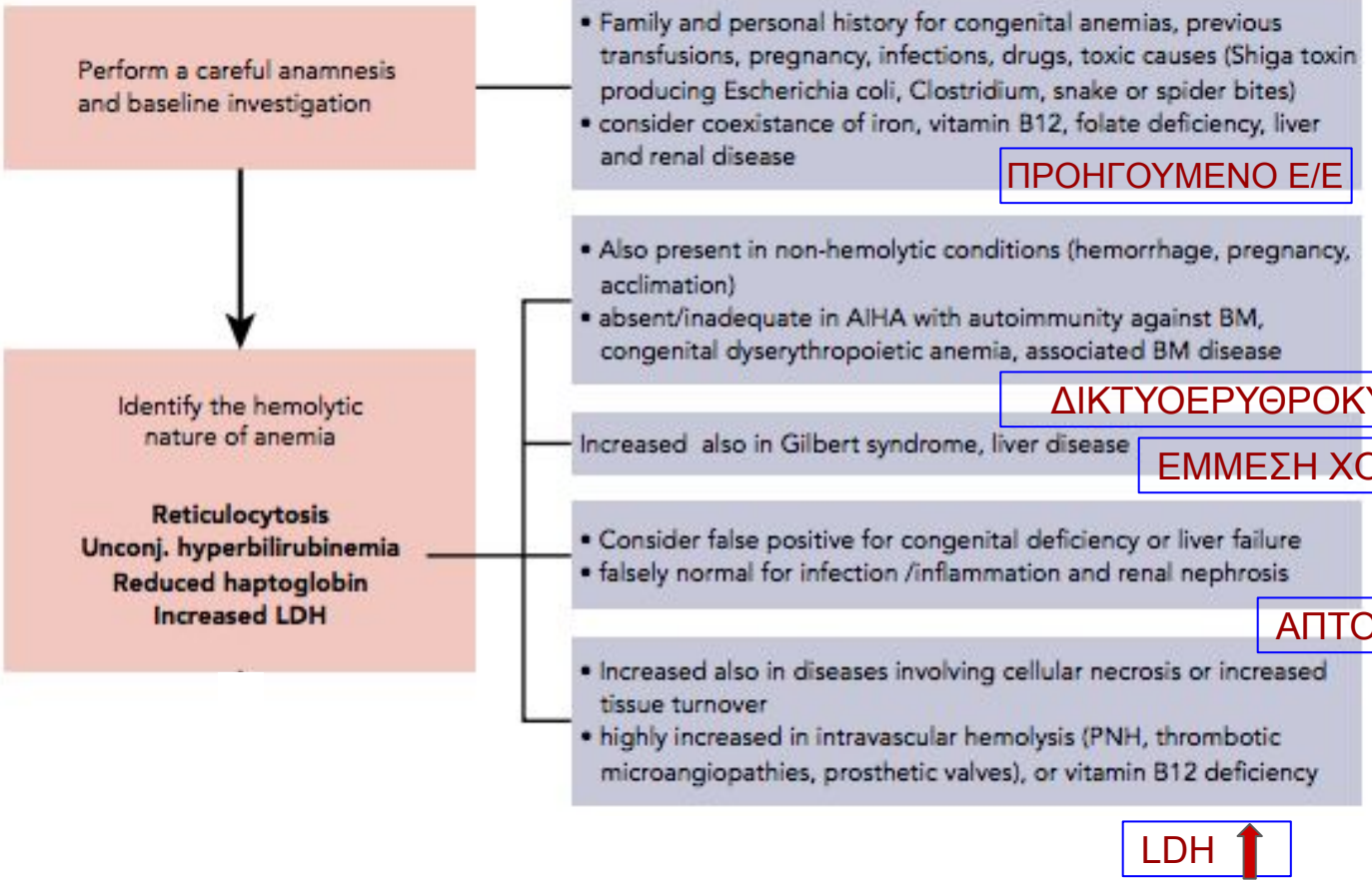


Initial Laboratory Evaluation:

- CBC with differential
- Reticulocyte count *
- LDH
- Bilirubin indirect **
- Haptoglobin
- Urinalysis (Hemosiderin) ***
- Smear

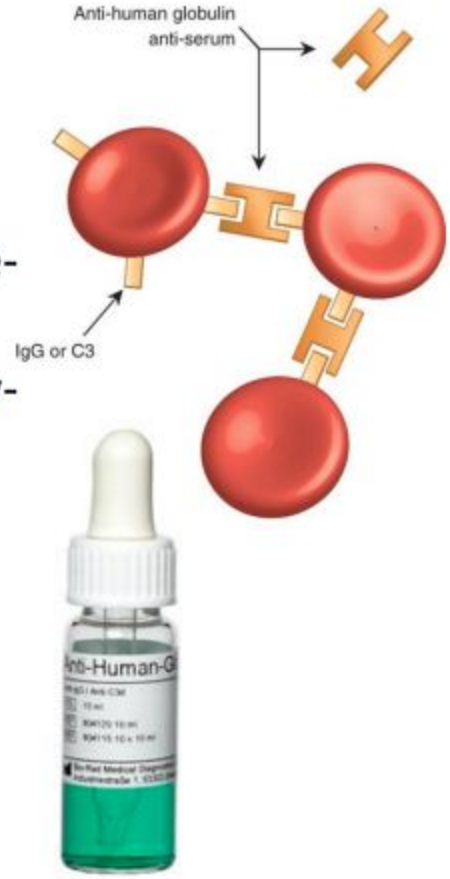


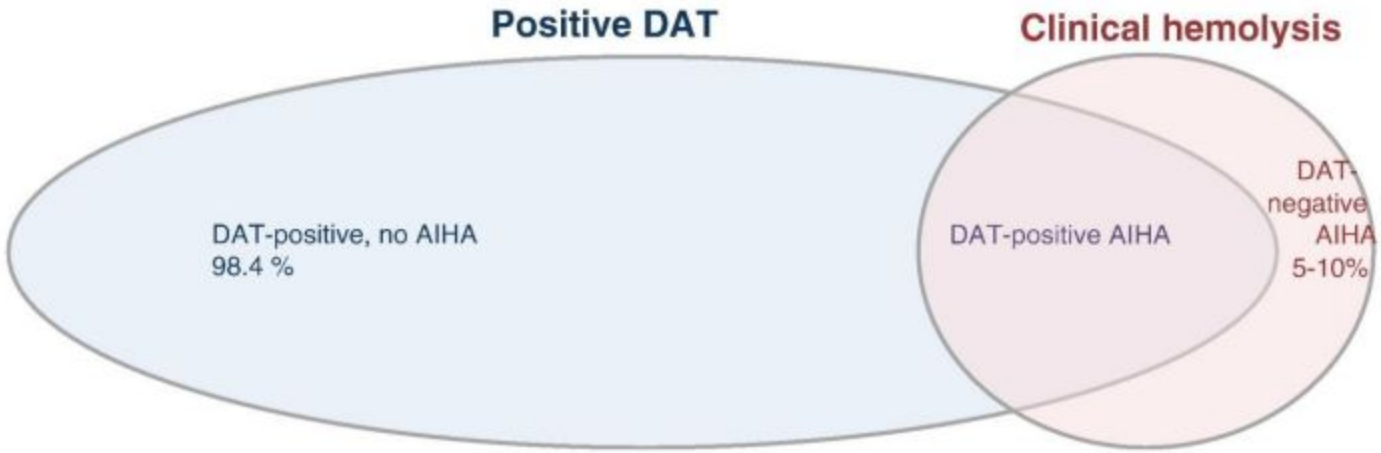
* Reticulocytopenia may occur in a minority of patients due to bone marrow infiltration by lymphoproliferative disease, immune attack of late-stage erythroid precursors or lag in marrow responsiveness.
** Bilirubin may be normal with mild hemolysis
***Suggestive of intravascular hemolysis


Perform a careful anamnesis and baseline investigation
Identify the hemolytic nature of anemia
Reticulocytosis
Unconj. hyperbilirubinemia
Reduced haptoglobin
Increased LDH
• Family and personal history for congenital anemias, previous transfusions, pregnancy, infections, drugs, toxic causes (Shiga toxin producing Escherichia coli, Clostridium, snake or spider bites)
• consider coexistance of iron, vitamin B12, folate deficiency, liver and renal disease
• Also present in non-hemolytic conditions (hemorrhage, pregnancy, acclimation)
• absent/inadequate in AIHA with autoimmunity against BM, congenital dyserythropoietic anemia, associated BM disease
Increased also in Gilbert syndrome, liver disease
• Consider false positive for congenital deficiency or liver failure
• falsely normal for infection /inflammation and renal nephrosis
• Increased also in diseases involving cellular necrosis or increased tissue turnover
• highly increased in intravascular hemolysis (PNH, thrombotic microangiopathies, prosthetic valves), or vitamin B12 deficiency
ΟΙΚΟΓΕΝΕΙΑΚΟ/ΑΤΟΜΙΚΟ ΙΣΤΟΡΙΚΟ
ΠΡΟΗΓΟΥΜΕΝΟ Ε/Ε
ΔΙΚΤΥΟΕΡΥΘΡΟΚΥΤΤΑΡΩΣΗ
ΕΜΜΕΣΗ ΧΟΛΕΡΥΘΡΙΝΗ
ΑΠΤΟΣΦΑΙΡΙΝΕΣ
LDH

Direct Antiglobulin Test (Coombs)

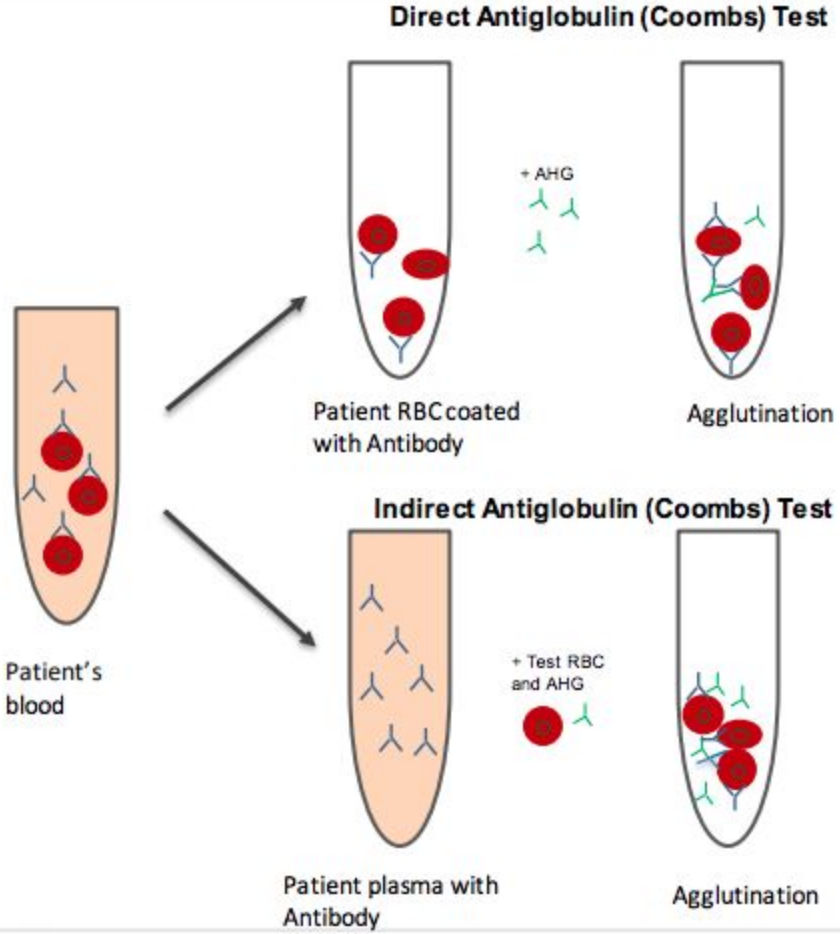
- Premise: Do RBC have surface bound IgG and/or complement (C3)? This helps to categorize hemolysis as immune dependent or immune-independent.
- Principle: antihuman globulin (AHG) agglutinates or clumps antibody-coated cells.
- Testing has poly-specific AHG containing both anti-IgG and anti-complement (anti-C3d).
- Detects 100-500 molecules of IgG or C3/RBC
- Caveats:
  - Fewer molecules can still lead to clinical significant hemolysis.
  - Will miss IgA or IgG4

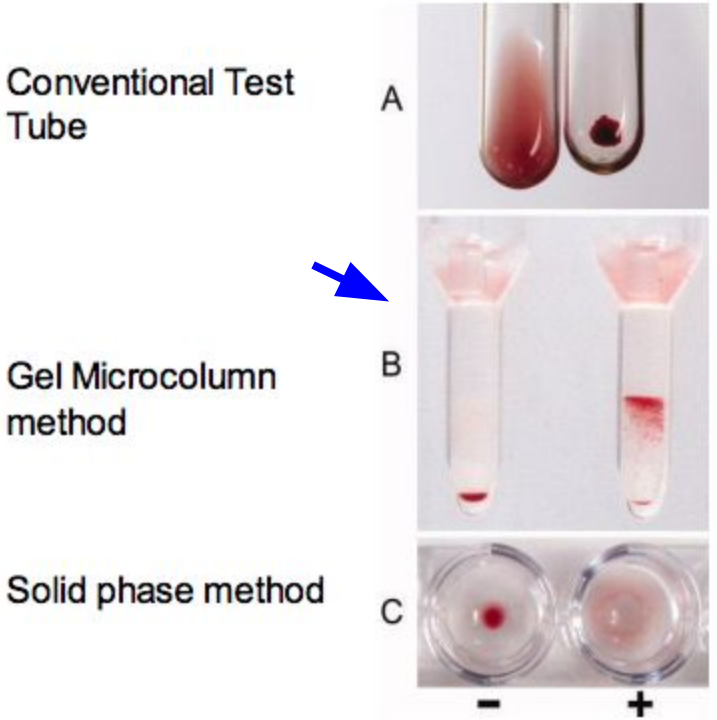




- The strength of a DAT has poor clinical correlation with severity of hemolysis.

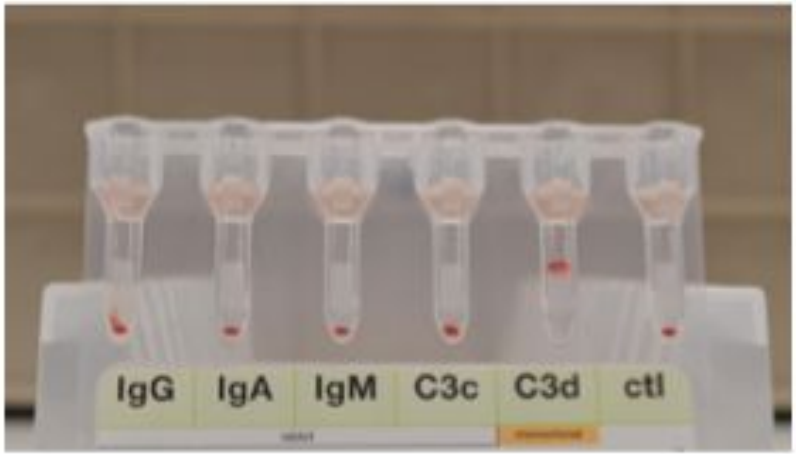
- A positive test does not mean hemolysis
  - 1:1000 to 1:14,000 healthy blood donors may have a positive DAT
  - DAT is positive in 7-8% of hospitalized patients


Direct Antiglobulin (Coombs) Test
+ AHG
Patient RBC coated with Antibody
Agglutination
Patient's blood
Indirect Antiglobulin (Coombs) Test
+ Test RBC and AHG
Patient plasma with Antibody
Agglutination


Conventional Test Tube
A
Gel Microcolumn method
B
Solid phase method
C
-
+

Serology Investigation

- DAT+
  - Anti-IgG only 20-60%
  - Anti-C3d only 7-14%
  - Both 24-63%

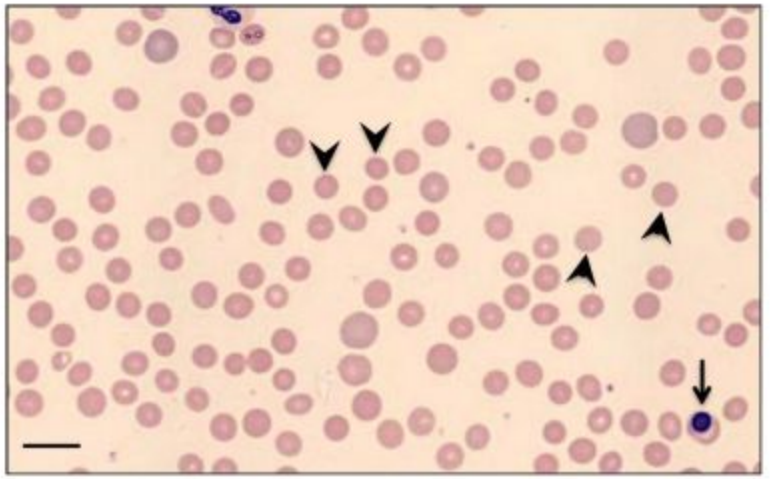


Autoimmune Haemolytic Anemia

- 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

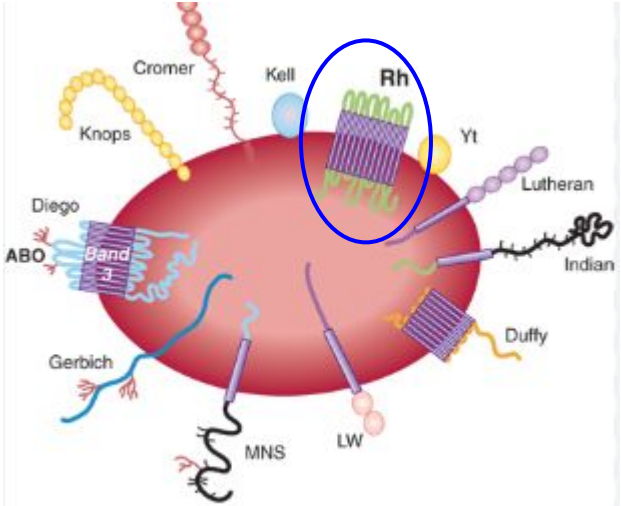
WAIHA

- The most common type of AIHA is mediated by warm reactive autoantibodies.
- Annual incidence: 1-3 cases per 100,000 persons.
- Median age of onset: 52 years
- Slight female predominance.
- IgG panagglutinating antibody directed against a public epitope: Rh system, glycophorin.
  - Optimally binds RBC at 37°C
  - May or may not bind complement.
  - Do not cause agglutination due to their small size.

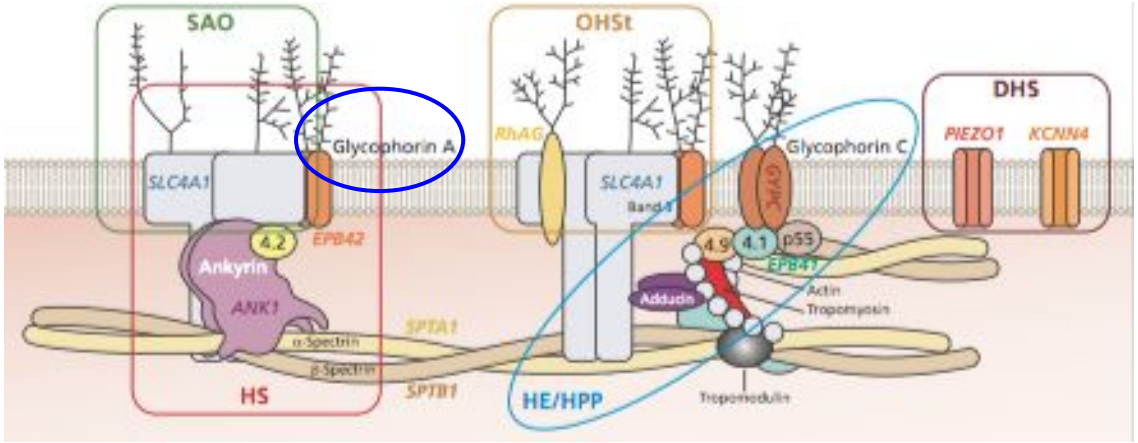


- Enhanced destruction of antibody coated RBCs, mediated by Fc receptor expressing macrophages in the spleen.
  - Partial phagocytosis results in formation of spherocytes.

RBC antigens : Carbohydrates, Proteins ..

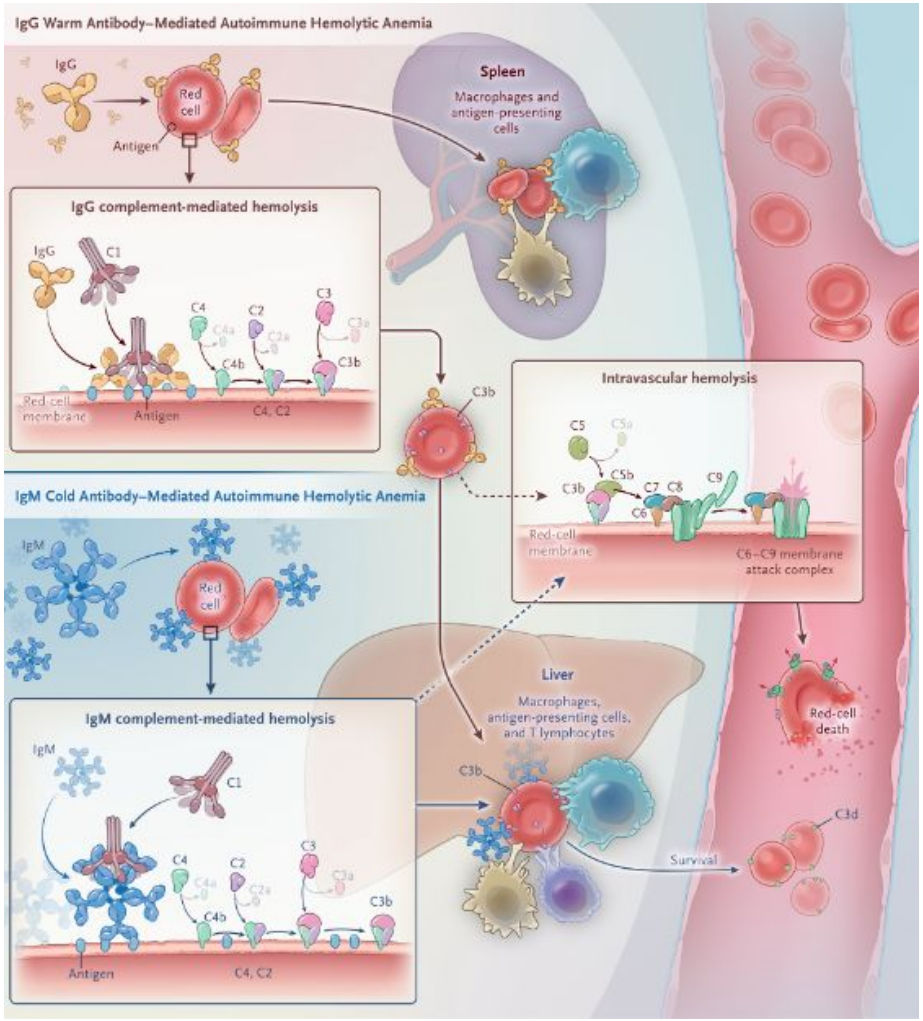


Source: H. Franklin Bunn, Jon C. Aster: Pathophysiology of Blood Disorders
www.accessmedicine.com



Mechanism of immune RBC lysis

- **Erythrocytes opsonized with immunoglobulin (typically IgG)** are destroyed by the **mononuclear phagocytic system**, mainly in the **spleen** and partly after transformation to **spherocytes**.
- **Complement-mediated hemolysis**, which can take place to a variable extent, occurs as **phagocytosis of C3b-labeled cells (extravascular hemolysis)** and occasionally even as **intravascular hemolysis**



WAIHA: Etiology

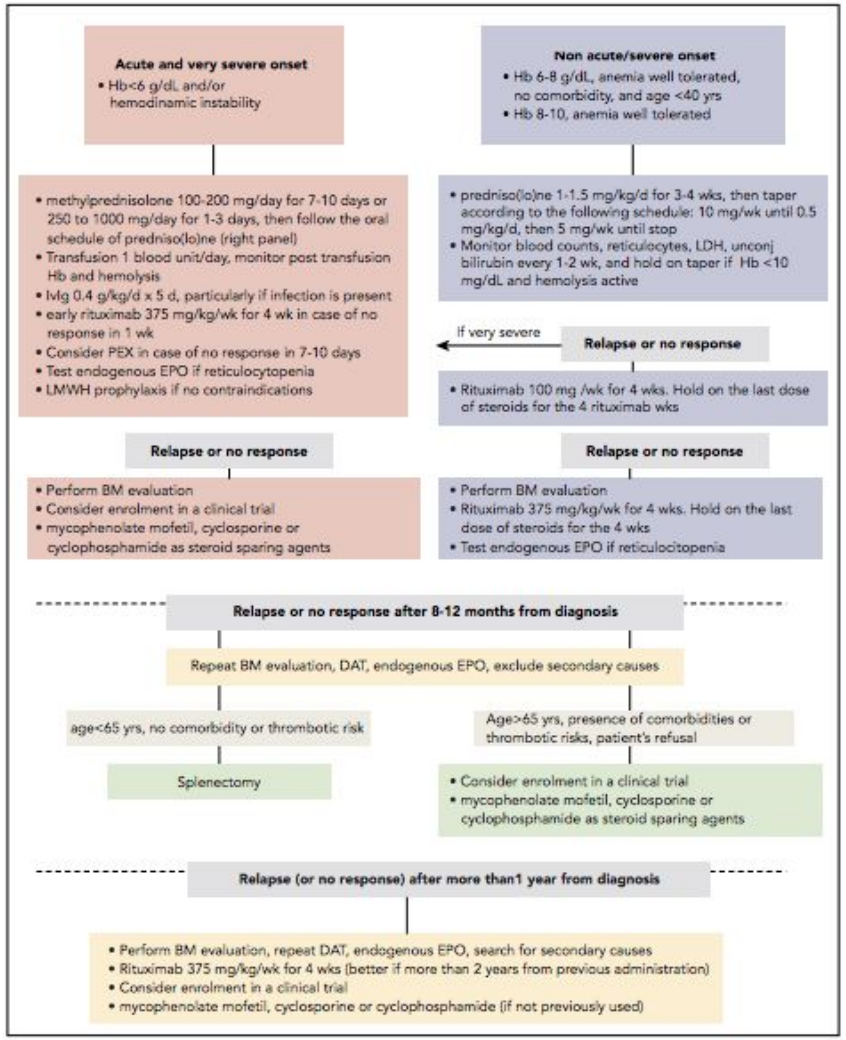
- Primary or Idiopathic
- Secondary:
  - Lymphoproliferative disorders (i.e. lymphoma, CLL)
    - Consider ordering flow cytometry
    - CT, bone marrow aspiration and biopsy may be indicated
  - Rheumatoid disorders (i.e. SLE)
    - Autoimmune panels in patients with signs and symptoms of rheumatologic disease.
  - Nonlymphoid neoplasms (i.e. ovarian tumors)
  - Chronic inflammatory diseases (i.e. ulcerative colitis)
  - Drugs (i.e. a-methyldopa)

Special Considerations

- AIHA is associated with increased risk of VTE (particularly WAIHA), further increased by certain associated conditions as well as treatments.
  - Patients who develop VTE with AIHA are high risk of recurrence
  - Trial off AC if stable remission, not receiving therapy or receiving minimal therapy.
- WAIHA may be exacerbated during pregnancy and in rare instances, maternal Ab can cross placenta and affect fetus.
- Evan's—co-occurrence of two or more cytopenias (platelets or neutrophils).
  - Very difficult to treat!

Don’t wait to Transfuse

- Transfusion can be lifesaving in the setting of WAIHA and severe anemia or unstable clinical/cardiac status
- Do not wait for ‘’compatible blood’’
- Do not wait for underlying alloantibodies to be worked up (several hours) when the anemia is severe and life threatening
- ‘’Least incompatible’’



Therapeutic algorithm

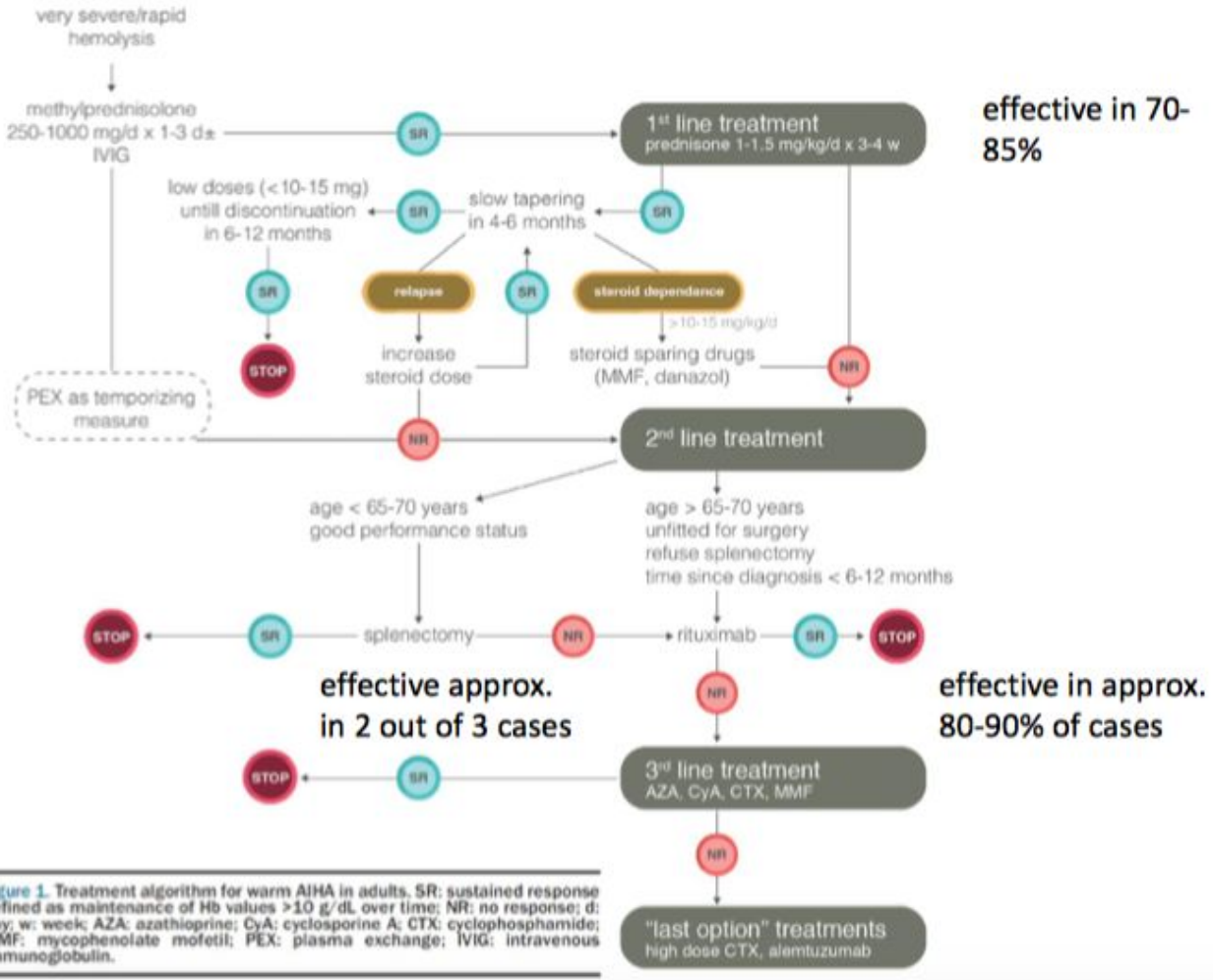
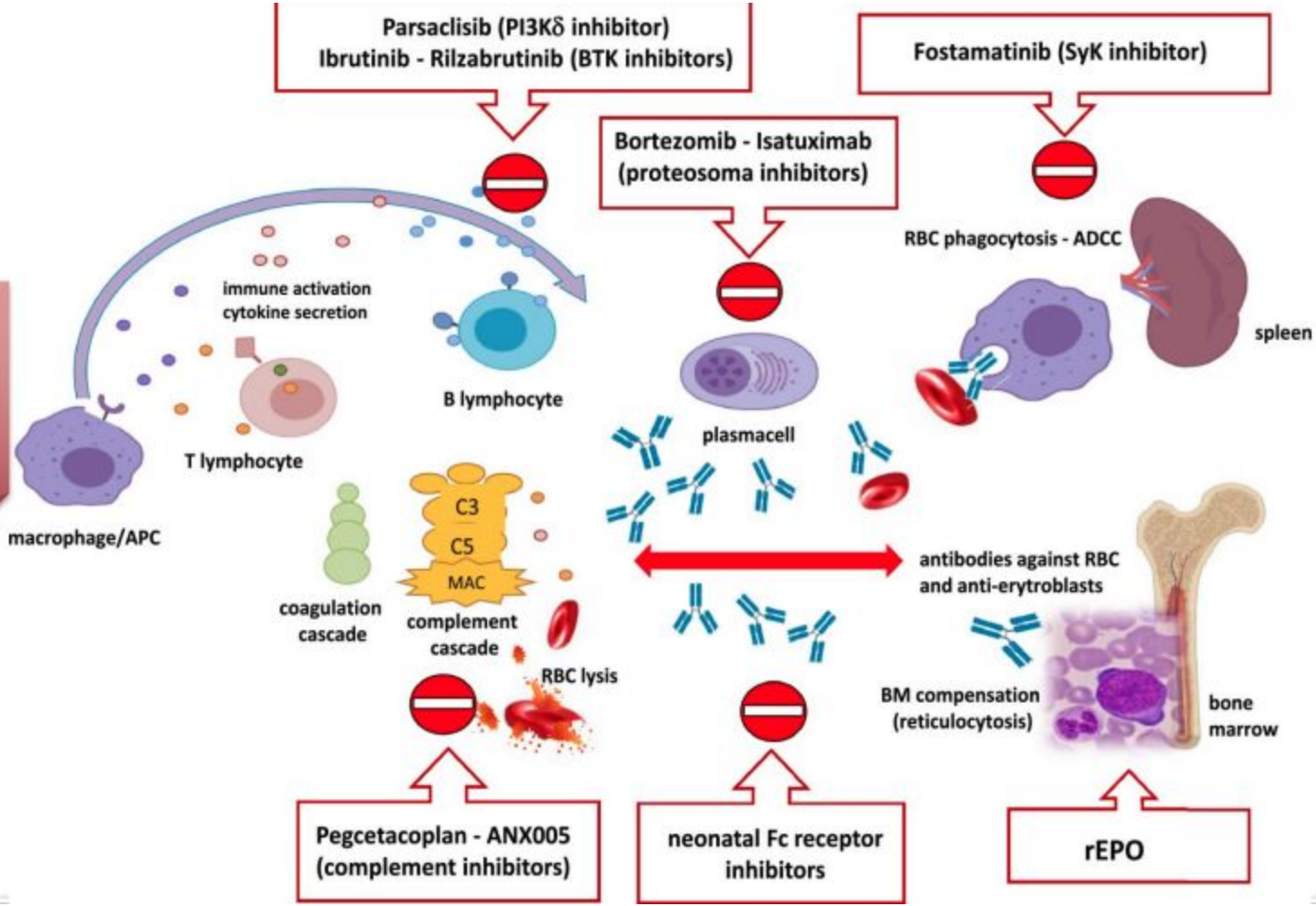


Figure 1. Treatment algorithm for warm AIHA in adults. SR: sustained response defined as maintenance of Hb values >10 g/dL over time; NR: no response; d: day; w: week; AZA: azathioprine; CyA: cyclosporine A; CTX: cyclophosphamide; MMF: mycophenolate mofetil; PEX: plasma exchange; IVIG: intravenous immunoglobulin.

Evolving Therapeutic Options



Ig's Thermal amplitude

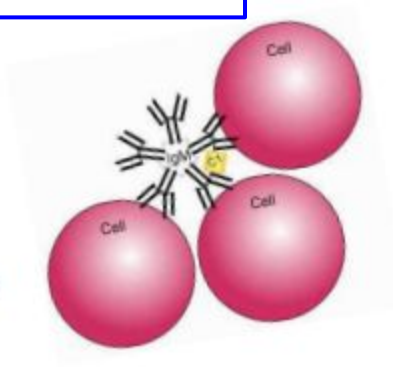
IgG ——————————IgA——————————————————IgM

37oC ——————————————————————— 4oC (Temperature)

Prevalance: 5-20 cases/million
Incidence: 0.5-1.9 cases/million/year (variation with climate)

RARE

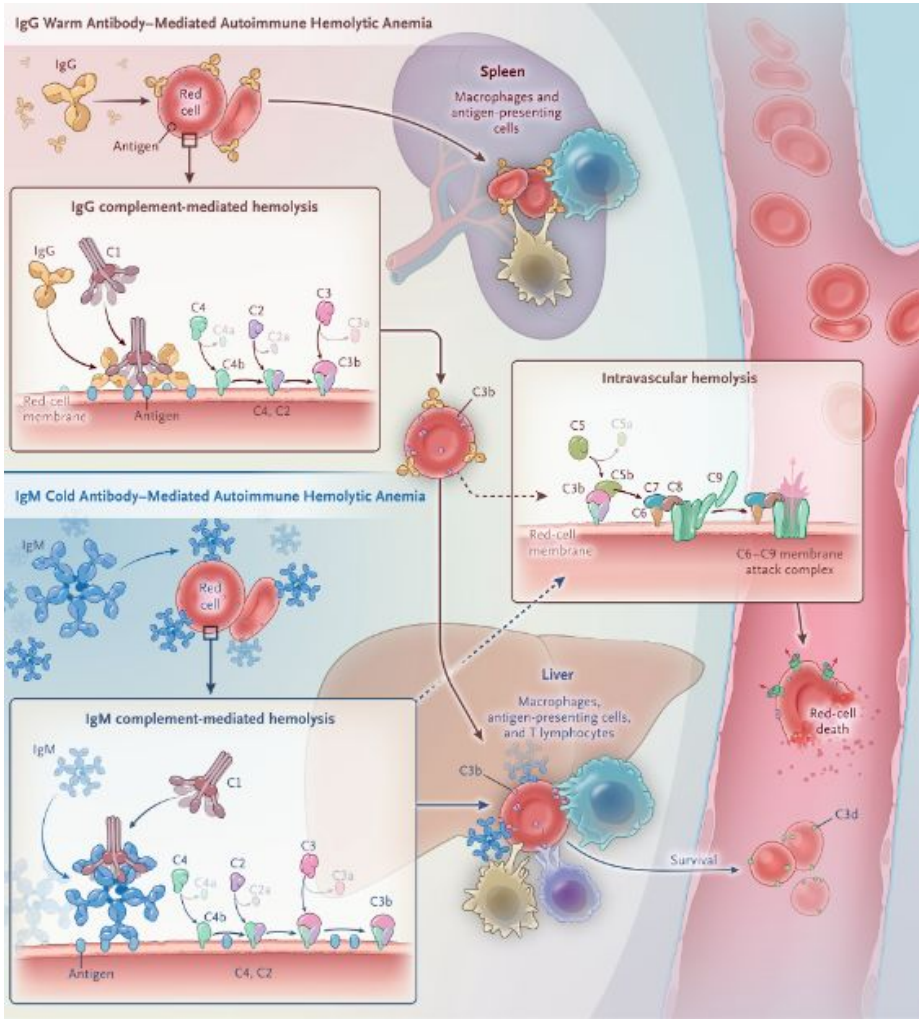
Cold Agglutinin Disease



- Cold reactive autoantibodies bind optimally to RBCs at temperatures <37° C.
- Typically, IgM isotype. Due to pentameric conformation, they can span the distance of several RBCs to cause direct agglutination.
- Mechanism: Fix complement
  - Clearance of C3b-coated cells by attachment to complement receptors in macrophages in spleen and Kupffer cells in liver.
  - Direct lysis of RBC by terminal complement sequence
- Titers >1:64 are considered clinically significant
- Thermal amplitude should be discerned
  - Highest temperature at which cold agglutinin reacts
  - More clinically significant than titer

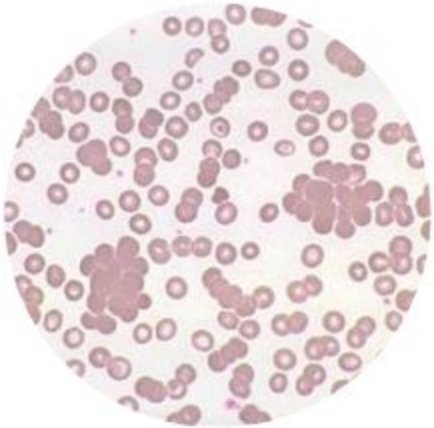
Mechanism of immune RBC lysis

- Autoantibody is typically **IgM** and the **hemolysis is complement-dependent**, mainly mediated by **extravascular hemolysis of C3b-opsonized cells in the liver**

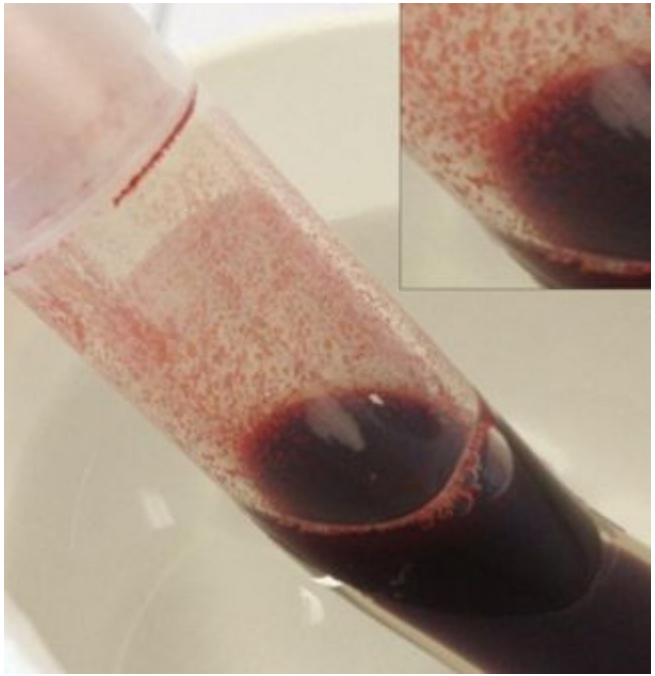


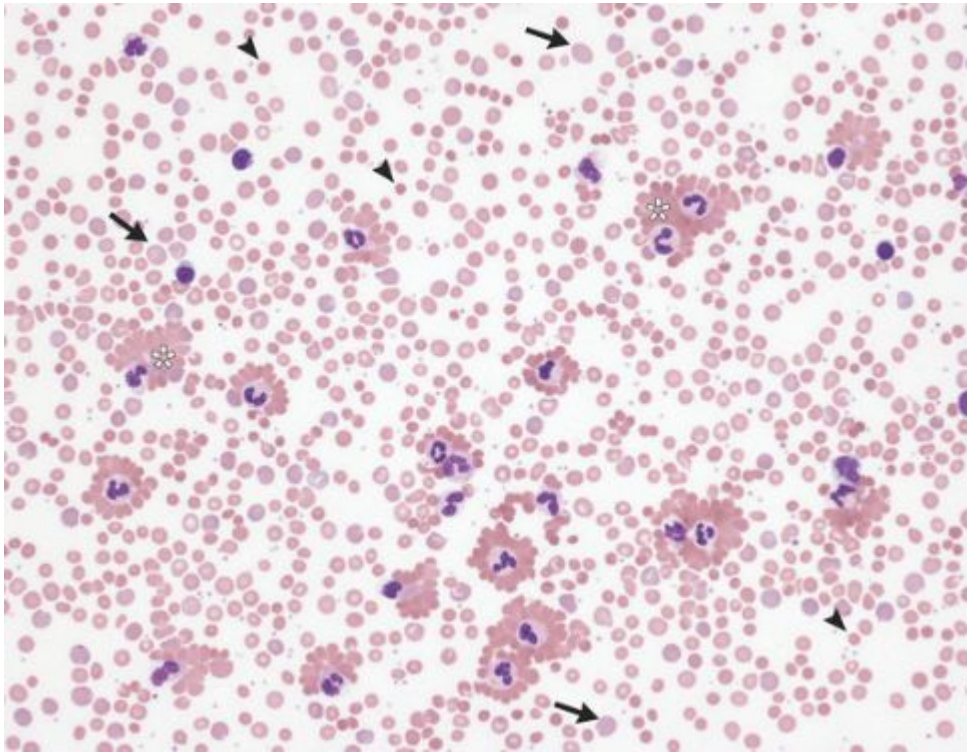
CAD

- Cold agglutinin most often directed against the Ii blood group
  - 90%
- Neonatal RBCs express i antigen
- After 18 months of age, RBCs express I antigen
- Smear: RBC agglutination
  - Cooling of blood during passage through acral parts of the body allows cold agglutinins to bind to the RBC leading to agglutination, complement binding, and hemolysis
  - Will abate with warming
- Spurious elevation in MCHC and MCV



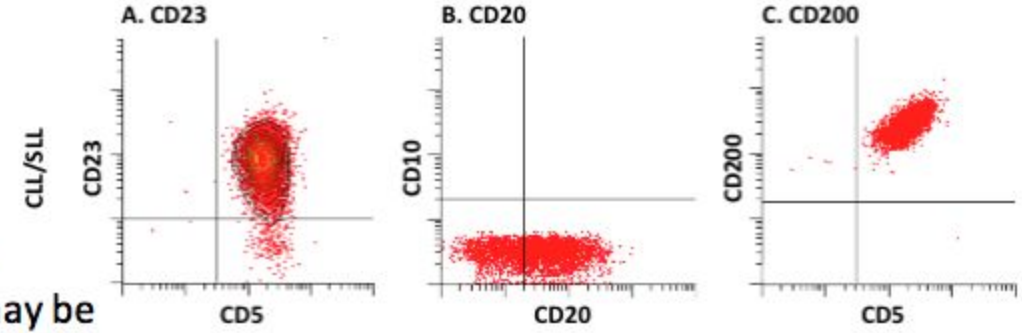
Cold Agglutinin Disease: Haematology View





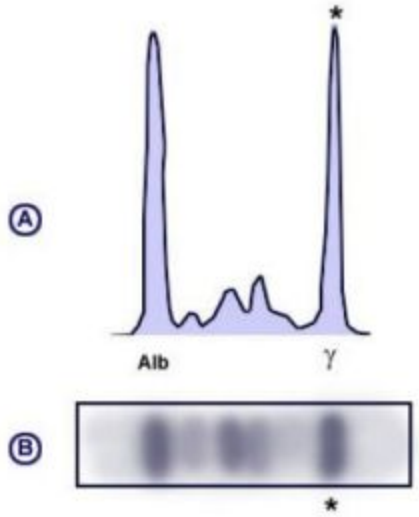
CAD: Etiology

- Primary/Idiopathic
  - Absence of an underlying disorder.
  - Clonal B lymphocyte proliferation may be detected



- Clonal B lymphocytes often found by flow even if the bone marrow morphology does not support lymphoma.

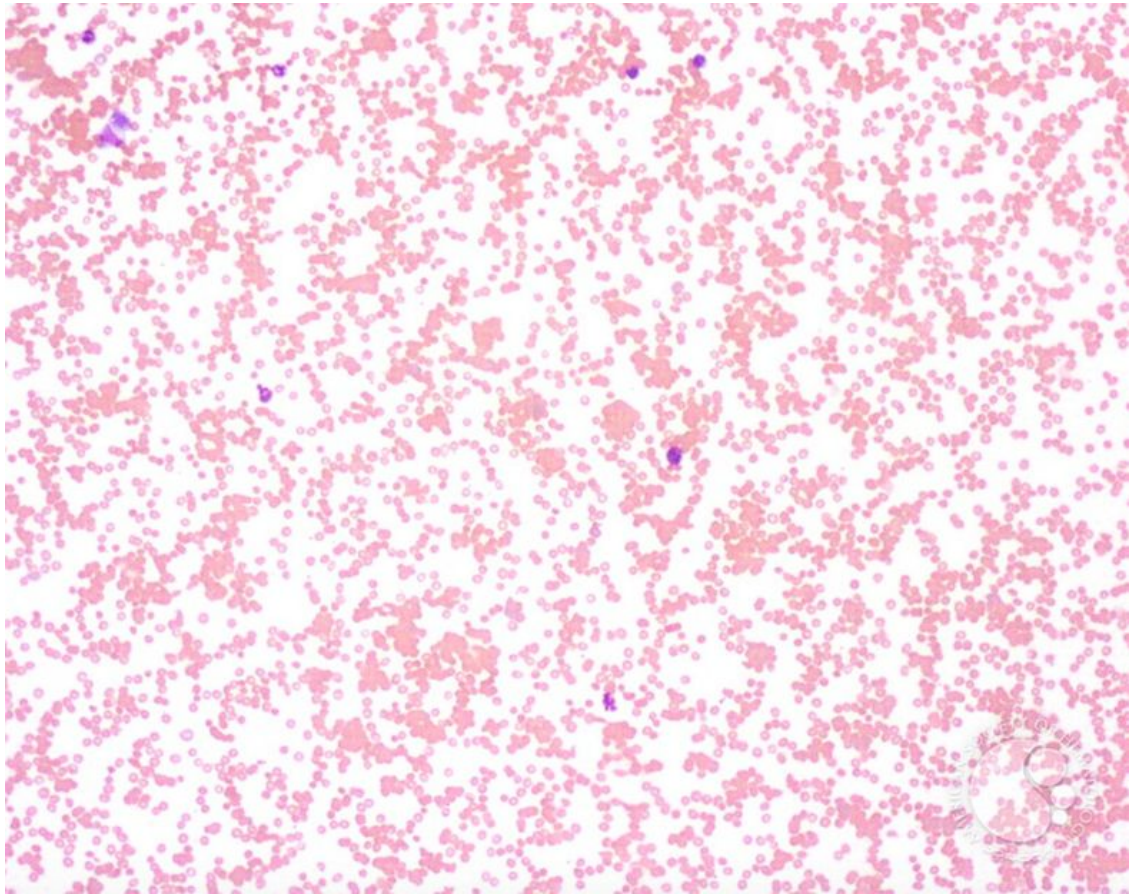
- Monoclonal band can be detected in majority of patients on SPEP
  - IgM k in >90% of cases



Cold Agglutinin Syndromes

Causes of Secondary Cold Agglutinin Syndromes		
Infections	Mycoplasma Pneumoniae Infectious mononucleosis Viral infections (rare)	Often Resolve spontaneous May need antibiotics
Autoimmune Disorders	SLE Rheumatoid Arthritis	Treat according to accepted standards
Lymphoid Malignancies	Non-Hodgkins Lymphhoma CLL Wadenstroms Macroglobulinemia Other lymphoproliferative diseases	Treat according to accepted standards

24 y.o boy with atypical pneumonia presents with the following CBC: Hb: 9,5mg/dl, MCV:115, MCH: 30, MCHC:34 RDW:18 RET# 300.000/µL, WBC: 11000/µL (N# 7000), PLT: 200000/µL



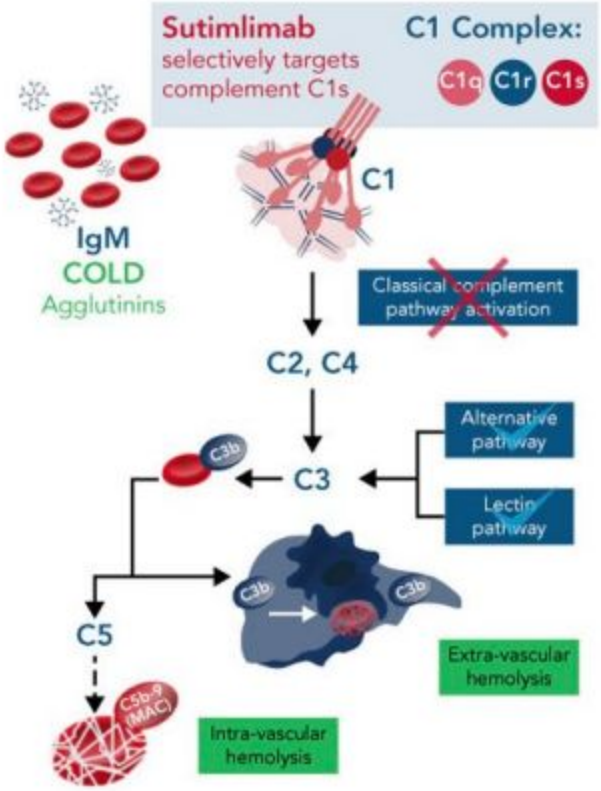
CAD: Treatment

- Anemia is often mild; no treatment needed.
- Avoid cold environments temperatures.
- If transfusion is necessary: transfuse through a blood warmer.
- Caution with bypass surgery or hypothermic surgeries.
- Treat underlying disease!
  - Lymphoma
  - Supportive care in case of infectious etiology
- Steroids and splenectomy are not generally effective.

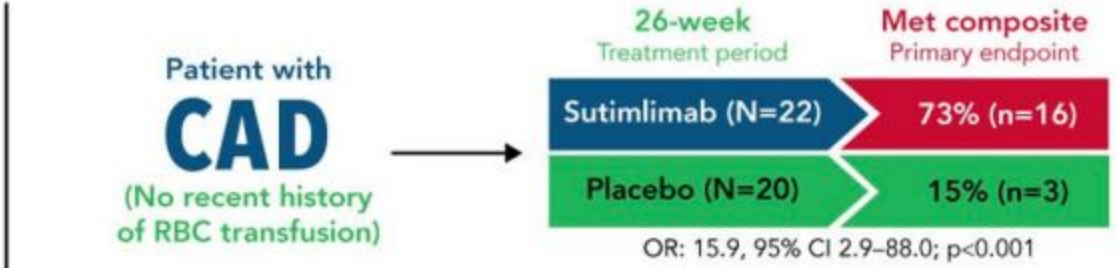
- For patients with primary CAD with or without detectable low grade bone marrow lymphoproliferative disorder, treatment is directed at patients with:
  - Symptomatic Anemia
  - Fatigue
  - Cold induced ischemic symptoms interfering with daily living

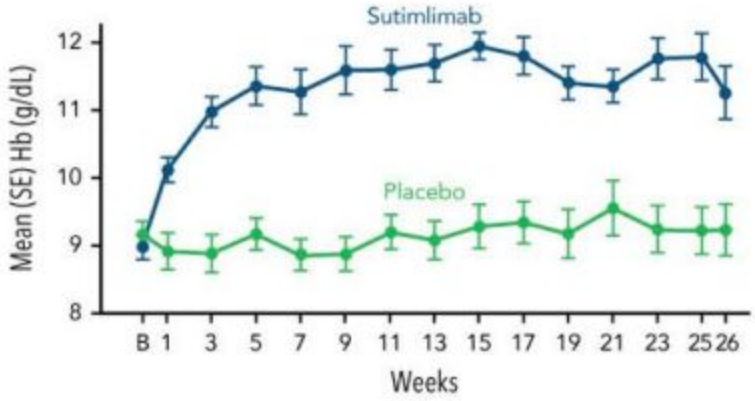
- Treatment aimed at the pathogenic B cell clone:
  - Rituximab containing therapy
  - Bortezomib
  - Ibrutinib

Sutimlimab: FDA approved 2022 for hemolysis in CAD



Patient with
CAD
(No recent history of RBC transfusion)





Early and sustained
Effects of sutimlimab on:

- ✓ Anemia
- ✓ Hemolysis
- ✓ Fatigue

No meaningful changes observed with placebo

CI, confidence interval; Hb, hemoglobin; IgM, immunoglobulin M; MAC, membrane attack complex; OR, odds ratio; RBC, red blood cells; SE, standard error

Alexander Röth et al. Blood

Paroxysmal Cold Hemoglobinuria

- Rare form of AIHA, primarily intravascular hemolysis
- PCH can occur at any age, but with the exception of adults with tertiary syphilis, nearly all cases affect young children.
- Now, often associated with acute viral infections
  - Mostly seen in pediatric population.
- Donath Landsteiner antibody biphasic hemolysin.
  - Complement fixing **IgG** directed against **P** antigen
  - It attaches to RBC and binds complement at < 37ºC
  - Activates complement as temperatures reach 37ºC

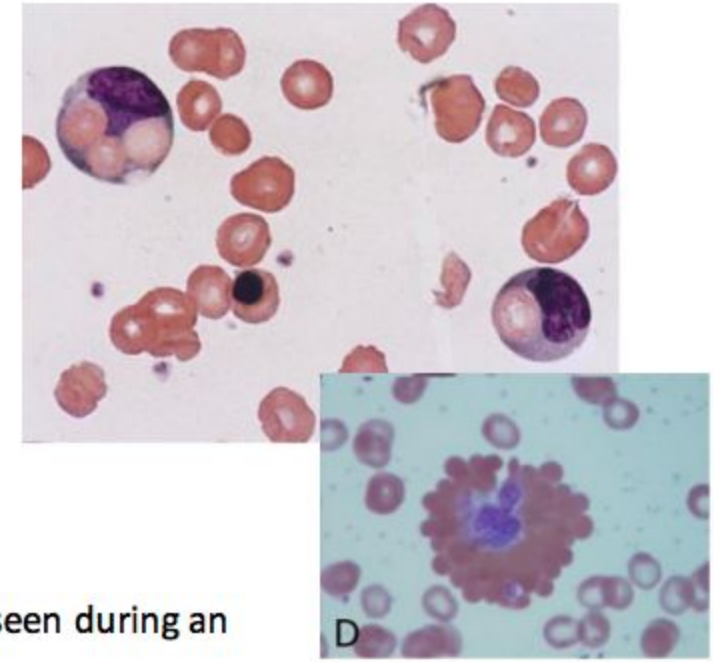
PCH: Clinical Features

- Antibody appears 7 - 10 days after the onset of the febrile illness
  - May persist for 6 to 12 weeks after
- Onset of hemolysis is shortly after cold exposure and hemolysis generally does not persist, once cold exposure ceases.
- Raynaud's phenomenon
- Urticaria

Signs and symptoms related to intravascular hemolysis
Dark urine shortly after cold exposure (min to hrs)
Abdominal cramping
Back/leg pain
Nausea/vomiting
Fever

PCH: Laboratory Features

Laboratory findings
Positive hemolysis labs (low haptoglobin, elevated LDH and indirect bilirubin)
Decreased serum complement levels
Positive DAT for C3d and negative for IgG during hemolytic episode
Positive Donath Landsteiner Test



Smear with agglutination

Spherocytes and erythrophagocytosis by neutrophils may be seen during an acute attack

PCH: Treatment

- ✓ Self-limited
- ✓ Supportive care
- ✓ Keep patient warm
- ✓ If transfusions are indicated, use a blood warmer.
- ✓ Hydration to prevent kidney injury from the intravascular hemolysis.
- ✓ Questionable use of steroids
- ✓ Splenectomy not useful

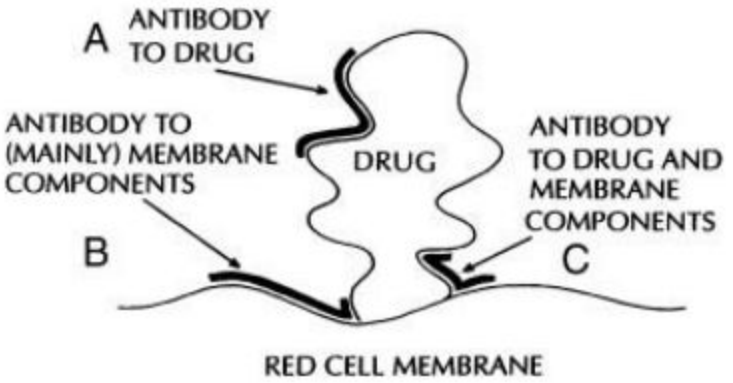
- ✓ In the case of recurrent or chronic symptomatic AIHA in whom cold avoidance and supportive measures are not enough:
  - ✓ Re-evaluate underlying conditions
  - ✓ Weak data for steroid use
  - ✓ Immunosuppressive agents:
    - cyclophosphamide,
    - azathioprine,
    - rituximab

Drug Induced AIHA

Over 70 drugs have been implicated:

Most cases will resolve after 1-2 weeks of ceasing the drug

Immune injury may be caused via different mechanisms:



Drugs associated with AIHA and/or + DAT

Antibiotics (~42%)

Amoxiclin
Amphotericin B
Ampicillin
Cefazolin
Cefotaxime
Cefotetan
Cefoxitin
Ceftazidime
Ceftizoxime
Ceftriaxone
Cefuroxime
Cephalexin
Cephalothin
Chloramphenicol
Ciprofloxacin
Erythromycin
Isoniazid
Levofloxacin
Mefloquine
Nafcillin
P-aminosalicylic acid
Penicillin
Piperacillin
Pyrimethamine
Quinidine
Quinine
Rifampin
Stibophen
Streptomycin
Teicoplanin
Temafloxacin
Tetracycline
Ticarcillin
Trimethoprim/ sulfamethoxazole

NSAIDS/analgesics (~16%)

Acetaminophen
Aspirin
Azapropazone
Diclofenac
Dipyrone
Ibuprofen
Methadone (positive DAT only)
Naproxen
Phenacetin
Sulfasalazine
Sulindac
Tolmetin

Chemotherapy (~13%)

Atezolizumab
Bendamustine
Carboplatin
Cisplatin
Cladribine
Fluorouracil
Fludarabine
Imatinib
Ipilimumab
Methotrexate
Nivolumab
Oxaliplatin
Pembrolizumab
Pemetrexed
Pentostatin

Other

Antazoline
Buthiazide
Carbimazole
Catechin
Chlorinated hydrocarbon insecticides
Chlorpromazine
Chlorpropamide
Cyclofenil
Cyclosporin
Diethylstilbestrol
Furosemide (positive DAT only)
Hydrochlorothiazide
Insulin
Methadone (positive DAT only)
Methotrexate
Nomifensine
Probenecid
Puerarin (Chinese herb)
Quinidine
Quinine
Radiocontrast medium
Tolbutamide
Triamterene
Trimellitic anhydride (used in dyes, resins

DAIHA: Treatment

Hemolysis can range from mild, asymptomatic or unrecognized hemolysis without anemia to fatal hemolytic reaction.

Cornerstone of treatment: discontinuation of the presumed offending drug.

Symptomatic drug-induced AIHA, can be treated with immunomodulatory therapies when it is not immediately clear whether the cause is drug-induced or autoimmune (without a drug), anemia is severe or there is insufficient time to observe for a response to drug discontinuation alone.

Differential diagnosis of reaction patterns of the direct antiglobulin test (DAT)

Reaction pattern	Differential diagnosis
IgG alone	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: hapten/drug adsorption type or autoantibody type
Complement alone	Warm antibody autoimmune hemolytic anemia with subthreshold IgG deposition
	Cold agglutinin disease
	Paroxysmal cold hemoglobinuria
	Drug-immune hemolytic anemia: ternary immune complex type
IgG plus complement	Warm antibody autoimmune hemolytic anemia
	Drug-immune hemolytic anemia: autoantibody type (rare)

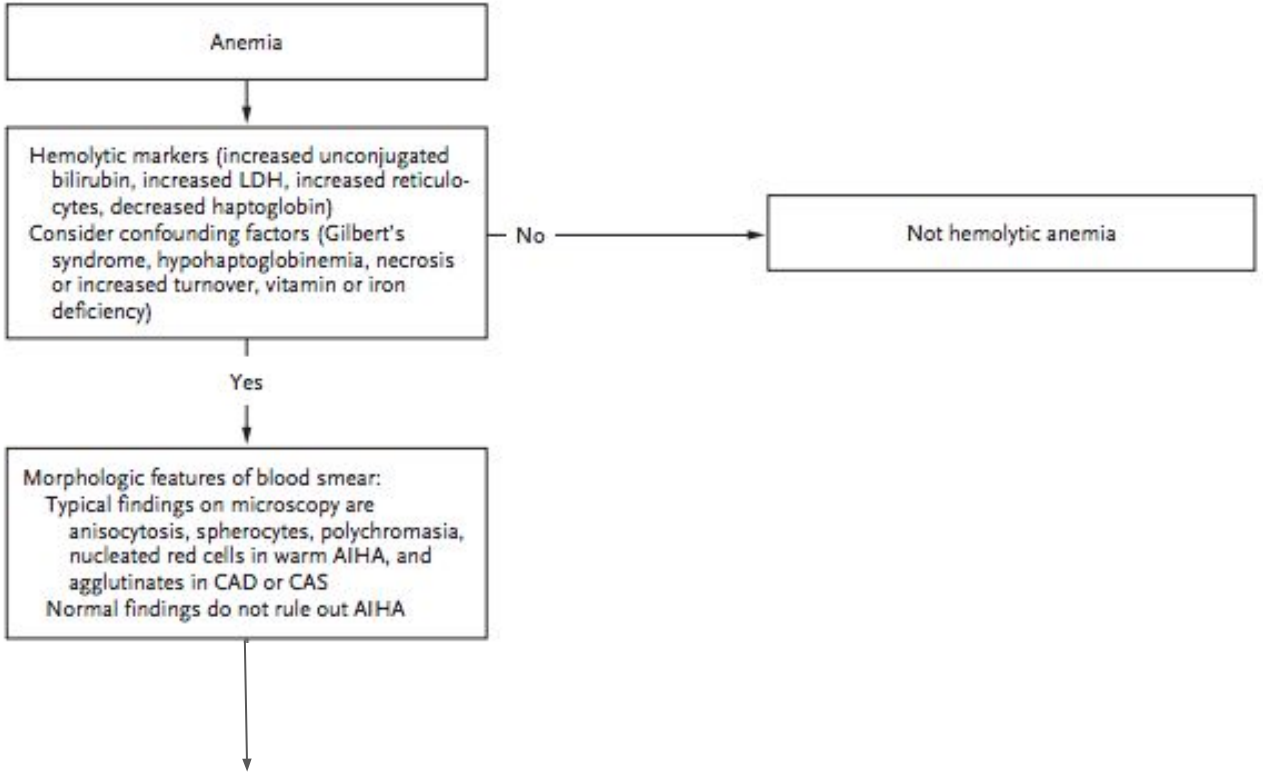
Review of AIHA Types

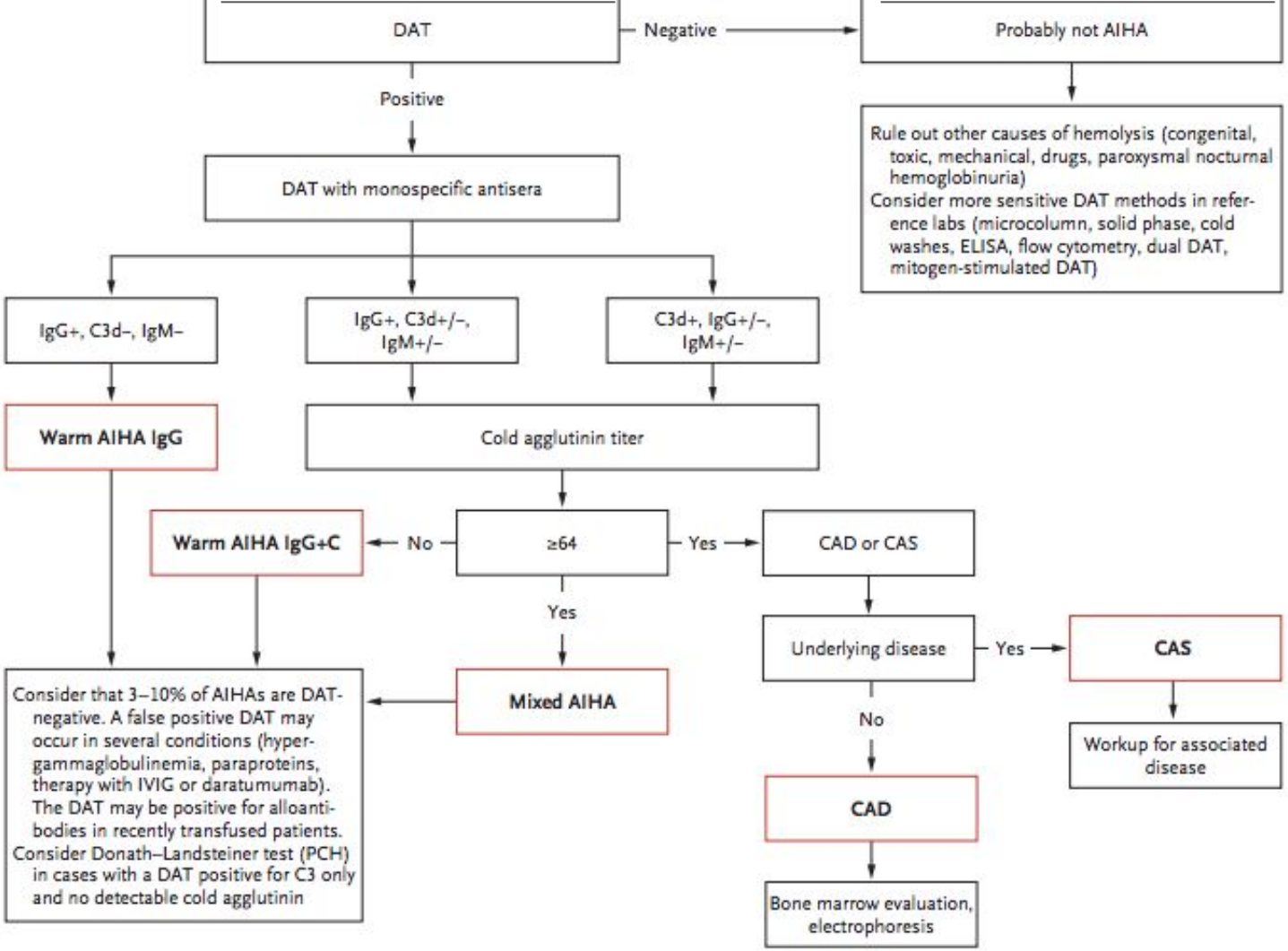
	WAIHA	CAD	PCH
Autoantibody Isotype	IgG	IgM (rarely IgG or IgA)	IgG (polyclonal)
Thermal Reactivity	Warm	Cold	Cold
Ability to fix complement	Variable	Yes	Yes
DAT	37˚C--positive for IgG ± C3	4˚C-neg for IgG; pos for C3 37˚C--neg for IgG, pos C3	4˚C—pos for IgG and C3 37˚C--neg for IgG , pos C3
Antigen	Rh, others	I or i	P
Site of hemolysis	Spleen	Intravascular	Intravascular
Etiology	Idiopathic Lymphoproliferative, autoimmune, malignancy, Drugs	Idiopathic Lymphoproliferative, autoimmune, malignancy	Syphilis, viral
First Line Therapy	Steroids	Avoidance of Cold	Avoidance of Cold
Second Line Therapy	Rituximab Splenectomy	Rituximab +/- Bendamustine	Steroids, immunosuppressive agents

Comparison between Cold Reacting-Antibody Diseases

	PCH	CAD	Cryoglobulinemia
Presentation	Childhood, rarely adults Dark, red urine after cold exposure Fever, chills, back or leg pain	Older adults, typically women > 70 y/o Anemia symptoms, acrocyanosis	Older adults, >60 y/o Palpable purpura, arthralgias, myalgias Chronic
Associated diseases	Secondary or tertiary syphilis Post viral (VZV, EBV, CMV, measles, mumps) Post bacterial (mycoplasma, E. Coli, Klebsiella, H. Flu) Post measles vaccine Autoimmune disorders Lymphoproliferative (lymphoma, CLL)	Lymphoproliferative (mainly lymphoma) Infection (mycloplasma, mononucleosis) Monoclonal IgM gammopathy Autoimmune disorders	Infections (hepatitis, HIV, fungi, parasites) Autoimmune disorders Inflammatory bowel disorders Neoplasms Cirrhosis Vaccines Familial syndromes
Clinical Findings	Jaundice Hemoglobineia, hemoglobinuria Raynaud's Urticaria Anemia Neutropenia	Anemia Livido reticularis Acrocyanosis Fatigue, weakness, dyspnea Hemoglobinuria Dysphagia with cold food	Vasculitis (small, medium) Macules, papules Arthralgias, myalgias Peripheral neuropathy Raynaud's
Hemolysis	Intravascular, biphasic DAT + (C3+ and IgG -) **both may be negative between episodes	Extravascular **Some intravascular with massive hemolytic crisis DAT + (C3+, IgG-) **sometimes IgG positive if there is warm component	No hemolysis
Characteristic of Antibody	Against "P" antigen Cold reacting IgG Polyclonal No agglutination Moderate titer <1:160 Binds RBC to fix complement Hemolysis with rewarming	Against "I" or "i" antigen Cold reacting IgM Monoclonal or polyclonal RBC agglutination High Titer >1:2000 Binds RBC and fixes complement Some cold agglutinins also cryoglobulins	Mixture antibodies and complement Cold reacting IgM, IgG, IgA or combinations **may be isolated light chains or Rh factor positive Monoclonal, polyclonal or BOTH Concentration varies (mg/dL rather than titers) Will precipitate when serum or plasma cooled Complement may be consumed with immune complexes Does not interact with RBCs

Diagnostic algorithm: Haemolytic Anemia




DAT
Negative
Probably not AIHA
Positive
Rule out other causes of hemolysis (congenital, toxic, mechanical, drugs, paroxysmal nocturnal hemoglobinuria)
Consider more sensitive DAT methods in reference labs (microcolumn, solid phase, cold washes, ELISA, flow cytometry, dual DAT, mitogen-stimulated DAT)
DAT with monospecific antisera
IgG+, C3d–, IgM–
IgG+, C3d+/–, IgM+/–
C3d+, IgG+/–, IgM+/–
Warm AIHA IgG
Cold agglutinin titer
Warm AIHA IgG+C
No
≥64
Yes
CAD or CAS
Yes
Underlying disease
Yes
CAS
Workup for associated disease
No
CAD
Bone marrow evaluation, electrophoresis
Mixed AIHA
Consider that 3–10% of AIHAs are DAT-negative. A false positive DAT may occur in several conditions (hypergammaglobulinemia, paraproteins, therapy with IVIG or daratumumab). The DAT may be positive for alloantibodies in recently transfused patients.
Consider Donath–Landsteiner test (PCH) in cases with a DAT positive for C3 only and no detectable cold agglutinin