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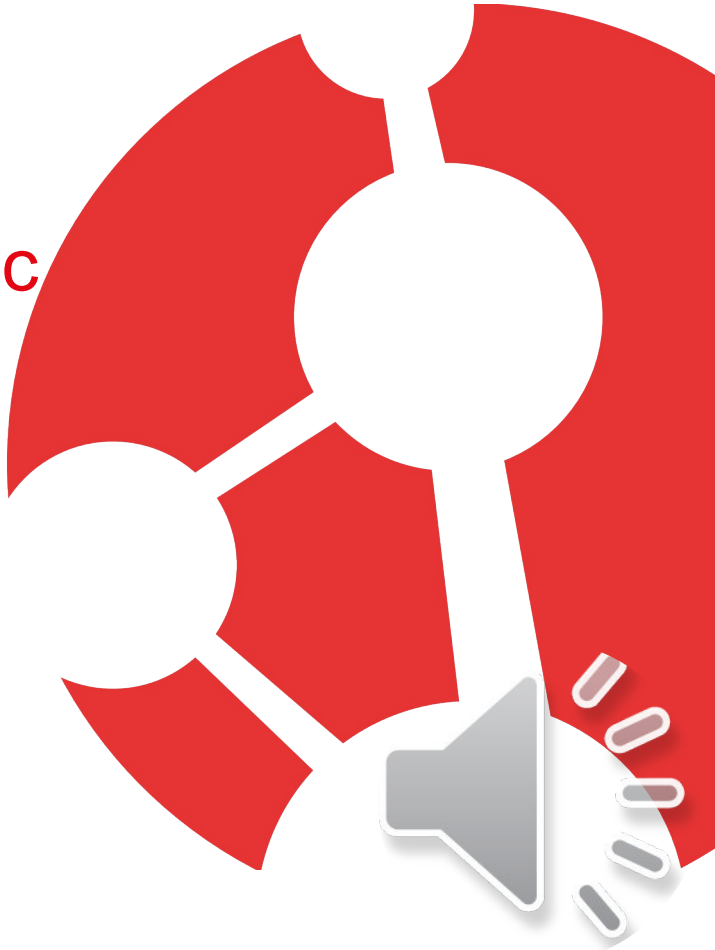
Acquired Hemolytic Anemia

15th November 2025

BHS Educational Course



Acquired Hemolytic Anemia

Narinx Justine – CHU Liège

15 November 2025

Introduction

- Regenerative anemia with macrocytosis:
  - Low Hb
  - High reticolocytes
  - High MCV
- Low Haptoglobine
- High bilirubine and LDH

Acquired Hemolytic Anemia

- Autoimmune hemolytic anemia (AHAI)
- Thrombotic microangiopathy (TMA)
- Paroxysmal Nocturnal hemoglobinuria
- Infections
- Medication and Toxicity
- Transfusion hemolytic reaction

Acquired Hemolytic Anemia

- **Autoimmune hemolytic anemia (AHAI)**
- Thrombotic microangiopathy (TMA)
- Paroxysmal Nocturnal hemoglobunuria
- Hemolytic anemia and infections
- Transfusion hemolytic reaction

AIHAI - Classification

Type of antibody

- Warm antibody AIHA
- Cold antibody AIHA
- Mixed antibody AIHA

Underlying disorder

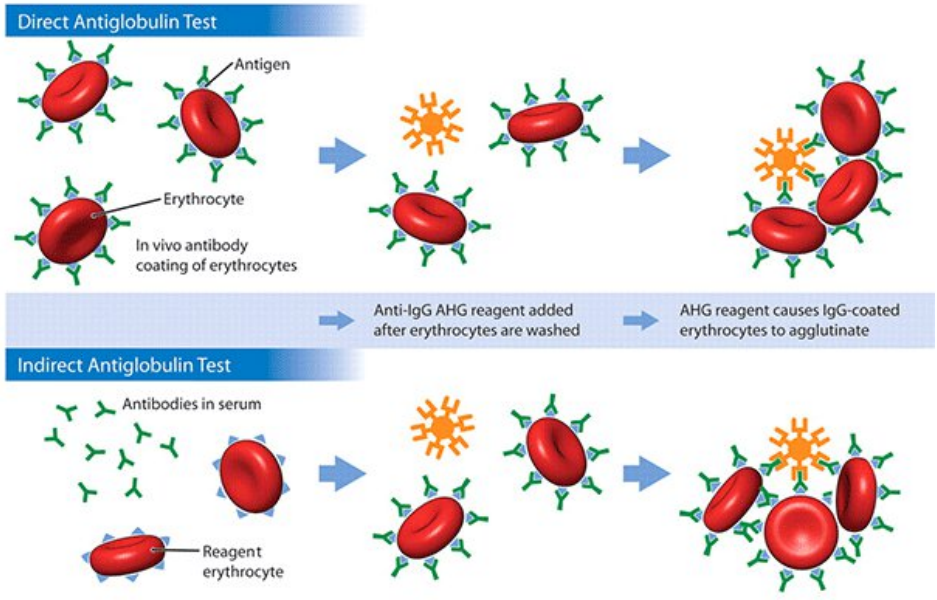
- Primary/Idiopathic AIHA
- Secondary AIHA

AIHI – Coomb's Test

Direct Coomb's Test

vs

Indirect Coomb's Test



Sagar Aryal, Microbe Notes 2022

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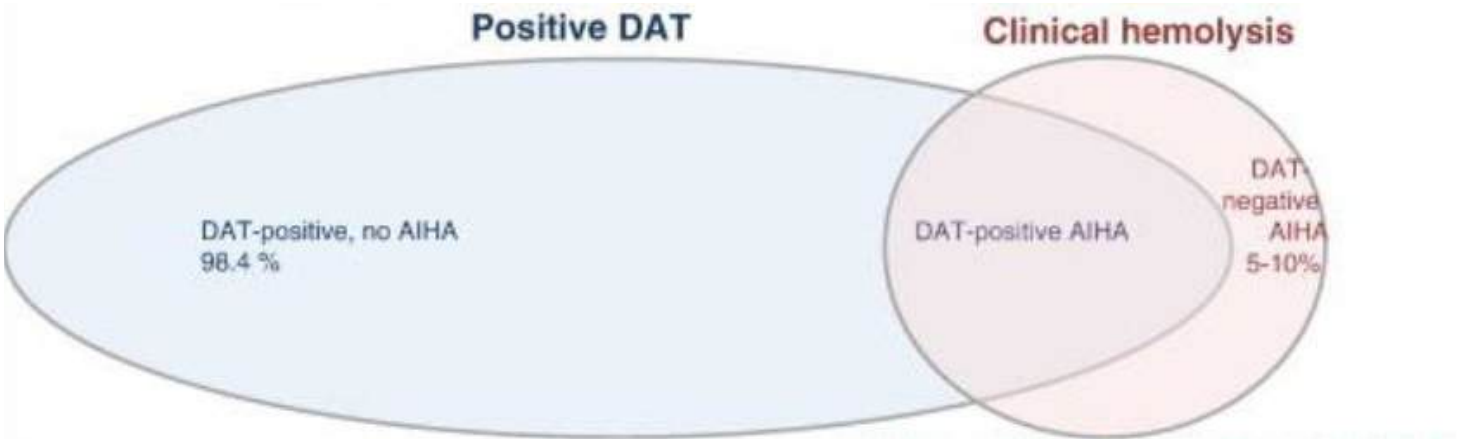
AIHA – Coomb's Test



Meulenbroek EM, et al.
Blood Rev 2015;29:369-76

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AIHA – Coomb's Test

- **DAT negative AIHA**

Table 3. Mechanisms involved in DAT-negative WAIHA

1. Erythrocyte-bound antibody below the limit of detection of standard DAT
Erythrocytes from healthy individuals have up to 35 molecules of IgG bound to their surface.
Standard DAT can detect >300-500 bound IgG molecules.
WAIHA can occur with as few as 70-434 bound IgG molecules.
2. Low-affinity IgG antibodies
Loosely bound antibodies are dislodged during the washing of erythrocytes or when samples are left standing at room temperature.
3. IgA antibodies
IgA antibodies may trigger phagocytosis and antibody-dependent cell cytotoxicity, resulting in hemolysis.
Standard anti–human globulin reagents do not have anti-IgA activity, as most polyspecific reagents contain a mixture of monoclonal anti-IgG and anti-C3d.
. Warm-reacting IgM and monomeric IgM antibodies
IgM antibodies reacting at warm temperatures and monomeric IgM may not fix complement.
Standard anti–human globulin reagents do not detect IgM. However, these antibodies will detect C3d if the IgM antibody fixes complement.

Table 4. Enhanced DATs

Name	Description	DAT-negative WAIHA detected
Column agglutination	Erythrocytes are placed on a column of beads suspended in diluent containing anti–human globulin reagent and centrifuged. Agglutination of antibody-coated erythrocytes results in cells failing to migrate in the bottom of the column. No cell washing is required, as serum/plasma is retained on top of the column.	Low-affinity autoantibodies
4°C Low-ionic-strength saline wash	Erythrocytes are washed with cold, low-ionic-strength saline to avoid removal of low-affinity antibodies.	Low-affinity autoantibodies
Polybrene	Polybrene induces aggregation of erythrocytes, which are dispersed by sodium citrate. If antibody is present, erythrocytes will not disperse.	Erythrocyte-bound antibody below limit of detection of standard DAT
Flow cytometry	Erythrocytes incubated with anti–human globulin reagent are tagged with fluorescence and examined by flow cytometry. Anti–human globulin reagents to IgG, IgA, and IgM are used.	Erythrocyte-bound antibody below limit of detection of standard DAT IgA antibody Erythrocyte-bound IgM and monomeric IgM antibody
IgA	Anti-IgA is used instead of anti-IgG and anti-C3d.	IgA antibody
IgM	Anti-IgM is used instead of anti-IgG and anti-C3d.	IgM warm antibody and monomeric IgM

Go RS, et al. Blood 2017;129:2971-9

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AIHA - Classification

Warm Antibody AIHA	Cold antibodies AIHA
37°C	4°C
Coomb's : IgG (+/- C3)	Coomb's : (IgM) + C3d
Splenic destruction	Hepatic destruction
Idiopathic, Autoimmune disease (SLE), CLL, Lymphoma, Medication	CAD, lymphoma, post-infectious (EBV, Mycoplasme pneumoniae)

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AIHA - Explorations

- **Autoimmune disease** : AAN, Antiphospholipid antibodies
- **Lymphoid hemopathy** : protein electropherosis & IF, lymphocyte immunophenotype, Bone marrow biopsy, TAP CT scan
- **Solid tumor**: TAP CT scan
- **Infection** : EBV, Mycoplasme pneumoniae, VHC, VHB, HIV, CMV, Treponema,…
- **Medications :** B-lactam antibiotics, AINS, quinine, purine nucleoside analogs (fludarabine and cladribine), and platinums (cisplatin and oxaliplatin)

AIHA - CAD

- AIHA with monoclonal cold agglutinin in the absence of any infection or lymphoma
- Process is driven by an underlying clonal B-cell proliferation wich not fulfill criteria for B-cell lymphoma

- Cold-induced symptoms : Accocyanosis, Raynaud, abdominal pain.

Level	Criteria
Required for diagnosis	Chronic hemolysis
	Monospecific DAT strongly positive for C3d
	Cold agglutinin titer ≥ 64 at 4 °C
	No overt malignant disease or relevant infection
Confirmative criteria not required for diagnosis	Monoclonal IgMκ in plasma/serum (or, rarely, IgG or λ phenotype)
	Ratio between κ- and λ-positive B-cells > 3.5 (or, rarely, <0.9)
	Cold agglutinin-associated lymphoproliferative disorder by histopathology
	MYD88 L265P mutation not found

Berensten et al Hemato 2022

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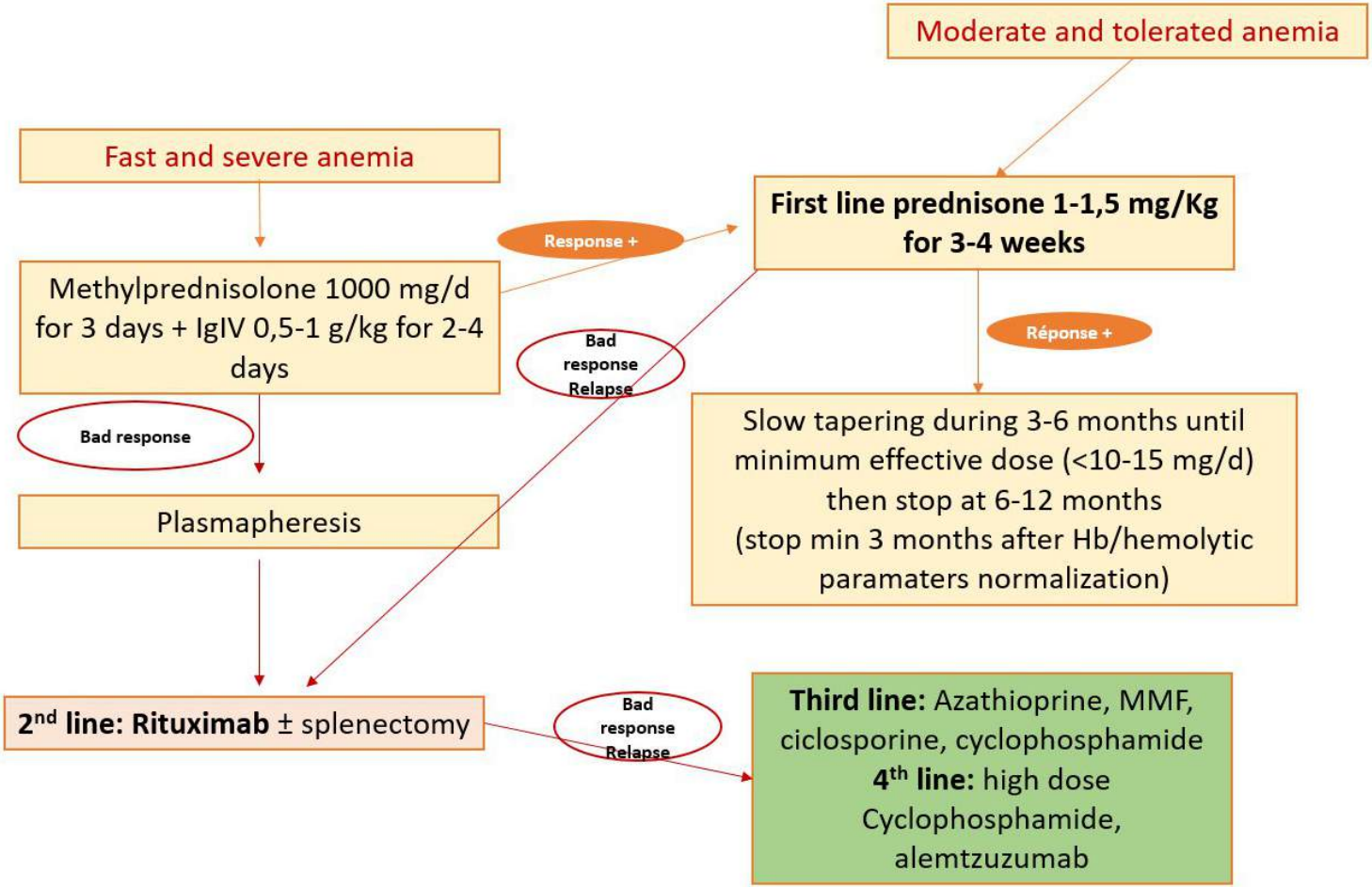
AIHA – Treatment : General recommendations

- Transfusion : only if untolerated severe anemia
- Hydration
- Folic acid supplementation and other vitamin deficiency
- HBPM prophylaxis during acute episode

AIHA – Treatment : Warm AIHA



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AIHA – Treatment : Cold AIHA

Transient CAS

Post-infectious

- Transfusion if needed
- Macrolide if Mycoplasme
- Favorable and spontaneaous evolution within few weeks

- Short course of corticotherapy (1mg/kg for 3-4 weeks) if severe anemia

CAS with NHL

Treatment of the NHL

AIHA – Treatment : Cold AIHA

No Steroids !
No Splenectomy !

Chronic CAD

- Avoid cold
- Prevention and treatment of febrile infection (vaccination)
- Warm transfusion (37°C)
- Folic acid

Acute episode

- Rituximab 375mg/m²/sem
- R-Bendamustine (4 cycles)

Relapse, bad response

- Sutimlimab (anti-C1s)
- Daratumumab / Bortezomib
- Ibrutinib

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Acquired Hemolytic Anemia

- Autoimmune hemolytic anemia (AHAI)
- **Thrombotic microangiopathy (TMA)**
- Paroxysmal Nocturnal hemoglobunuria
- Hemolytic anemia and infections
- Transfusion hemolytic reaction

Thrombotic Microangiopathy

- **Thrombotic thrombocytopenic purpura (TTP)** : ADAMTS 13 deficiency :
  - Congenital (ADAMTS 13 gene mutation) : 35% of pediatric cases and <2% of adult cases
  - Acquired (anti-ADAMTS13 Antibodies): 65% of pediatric case and 80% of adult cases
- **Hemolytic uremic syndrome (HUS)** :
  - Typical (Shiga-toxin producing E. coli)
  - Atypical : complement dysregulation (congenital or acquired)
- **Secondary TMA**
  - Solid organ or hematopoietic stem cell transplantation
  - Medication (clopidogrel, cyclosporine, tacrolimus, quinine, gemcitabine mitomycine,…)
  - Pregnancy
  - Auto-immune disease (anti-phospholipid syndrome, LES,…)

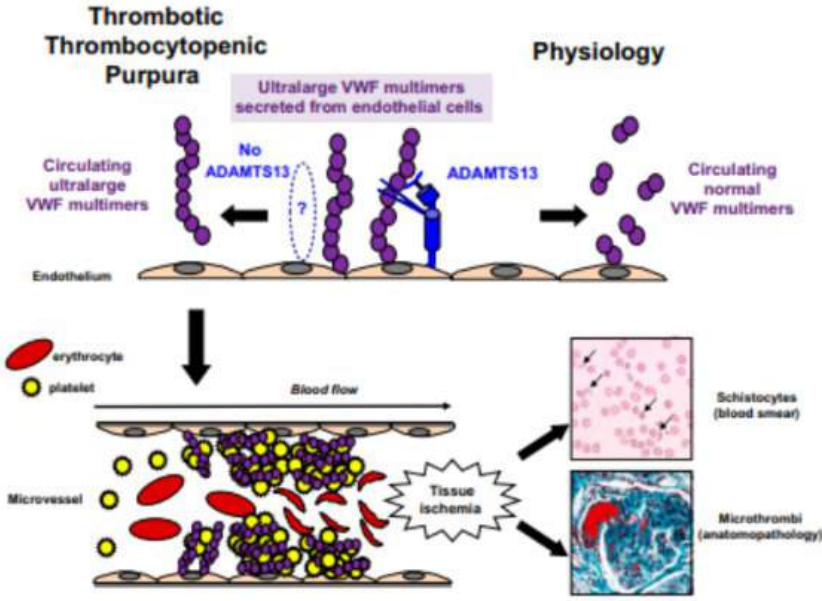
TTP - Physiopathology

↘ ADAMTS13 (congenital or auto-Ab)

- ↘ Von Willebrand factors multimers destruction
- Platelets aggregation and consumption

- Microvascular thrombi : tissular hypoxemia
  - MOF
- Bleeding

Ⓟ High mortality without treatment (>90%)



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TTP – Diagnosis

- **Nonimmune hemolytic anemia**
  - ↗ bilirubin and LDH, ↘ Haptoglobin
  - Schistocytes
  - Coombs negative
- **Thrombopenia :**
  - Often <20 000/mm³
  - Bleeding tendency
- **± CNS abnormalities :**
  - Coma, convulsion, focal deficit
- **± Renal abnormalities :**
  - Microscopic hematuria, proteinuria, oligo-anuria

/!\ Classical Pentade only in 7% of cases
GI symptoms in >1/3 of cases

TTP – Diagnosis

- **Nonimmune hemolytic anemia**
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- **± Renal abnormalities :**
  - Microscopic hematuria, proteinuria

è **ADAMTS 13 <10%**
But results within 24-48h

TTP – Plasmic score

Parameter	Result	Score
Platelet count	<30K	1
Creatinine	<2.0	1
INR	<1.5	1
MCV	<90	1
Presence of hemolysis variable	Either: -Retic>2.5% -Undetectable hapto-globin or -iBili>2 mg/dL	1
Abscence of active cancer		1
No prior stem cell or organ transplant		1

Severe deficiency in ADAMTS13 frequency:

0-4 : Low (0-4%)

5: Intermediate (5-24%)

6-7: High (62-82%)

TTP -Treatment

1. Plasmapheresis:

- Remove anti-ADAMTS 13 auto-Ab
  Contains non-inhibited ADAMTS13
- 1,5 plasma volume 1x/d (2x if no clinical improvement)
- Until platelets normalization > 150 000/mm³ during 2 days + clinical improvement + LDH diminution,

2. Corticostéroïdes

- 1 - 1.5mg/kg/d
- Until ADAMTS13 >20%

3. Rituximab

- 375mg/m² x4, every 2-3 weeks
- Not in emergency

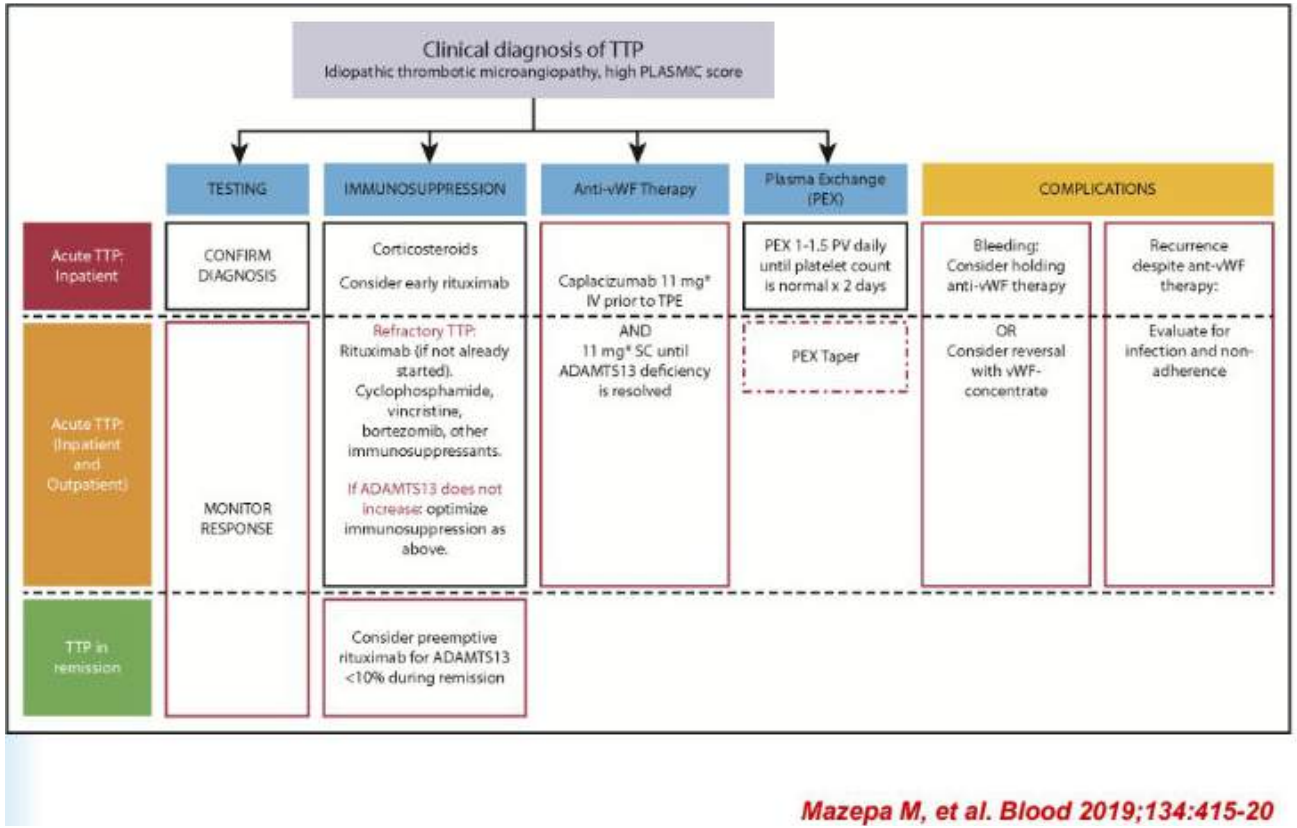
4. Caplacizumab

PTT -Treatment

4. Caplacizumab

- Anti-Von Willebrand F Ab ☾ inhibit platelets interaction
- D1: 10mg IV 1x before 1st plasmapheresis, then 10mg S.C. after
  Days after : 10mg S.C. after plasmapheresis
  During 30 days after last plasmapheresis (and ADAMTS13 >20%)
- Faster platelets normalization
- Improve OS
- ↗ Risk of bleeding

PTT -Treatment



Mazepa M, et al. Blood 2019;134:415-20

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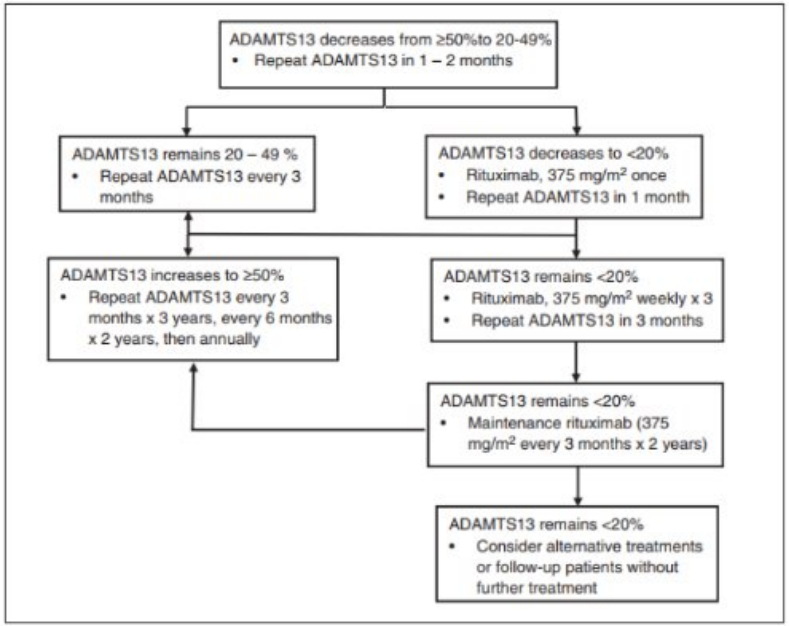
TTP –Treatment : General recommendations

- Erythrocytes transfusions : severe/symptomatic anemia
- Platelets transfusion : severe bleeding or invasive interventions
- Identify and treatment of triggering factor
- Folic acid supplementation

TTP – Follow up



Coppo P, et al. Res Pract Thromb Haemost 2018;3:26-37

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Hemolytic uremic syndrome (HUS)

- Nonimmune hemolytic anemia + schistocytes
- Thrombopenia
- Renal involvement : hematuria/proteinuria ☾ Acute kidney failure with oliguria
- HTA
- ± neurological symptoms

Typical HUS

- Shiga-toxin producing E. Coli (gastro-intestinal or urinary)
  - often bleeding diarrhea
- Pneumocoque

- Symptomatic treatment : hydro-electrolytic management, HTA, dialysis, transfusion, folic acid supplementation …
- If Shiga-toxin producing E. Coli: no antibiotherapy (increase toxin liberation)

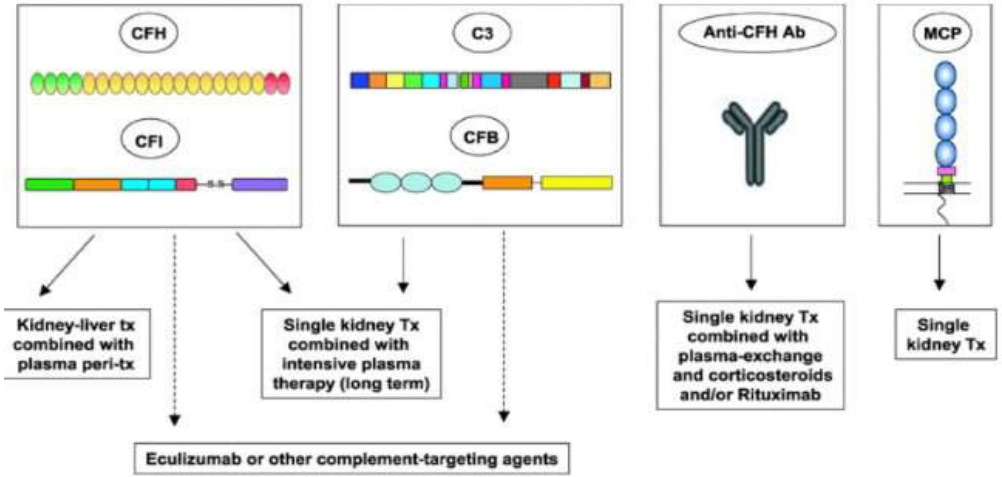
Atypical HUS

- Congenital or auto-immune defect in the complement cascade regulation
  - complement activation
- Often following infection
- Treatment : plasmapheresis, Eculizumab, Rituximab, steroids (Kidney transplantation)

Factor mutated	Frequency
Complement factor H (CFH)	20-30%
Membrane cofactor protein (MCP = CD46)	5-15%
Complement factor I (CFI)	4-10%
Thrombomodulin (THBD)	3-5%
C3	2-10%
Complement factor B (CFB)	1-4%

Auto-antibodies	Frequency
Anti-CFH	6%

Atypical HUS - Treatment



Noris M, et al. Am J Transpl 2010;10:1517-23

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Acquired Hemolytic Anemia

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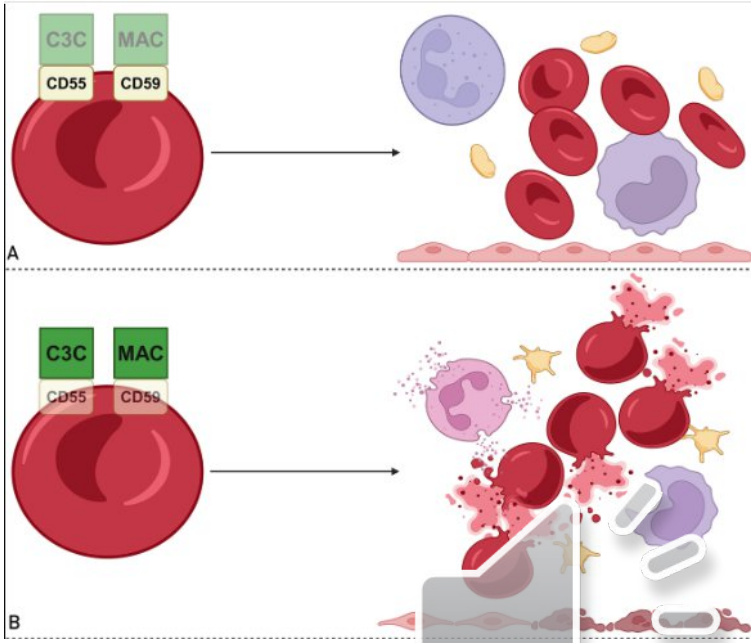
- Thrombotic microangiopathy (TMA)
- **Paroxysmal Nocturnal hemoglobinuria**
- Infections
- Medication and Toxicity
- Transfusion hemolytic reaction

Paroxysmal Nocturnal hemoglobinuria (PNH)

- Acquired disorder in hematopoietic stem cells
- Mutation in PIG-A gene
  - reduced or absent GPI-anchored proteins on cell membrane
- Loss of the GPI-linked complement inhibitors, CD55 and CD59, on red blood cells
- Intravascular complement driven hemolysis and thromboembolic risk



Godby, Richard C. et al.
Mayo Clinic Proceedings

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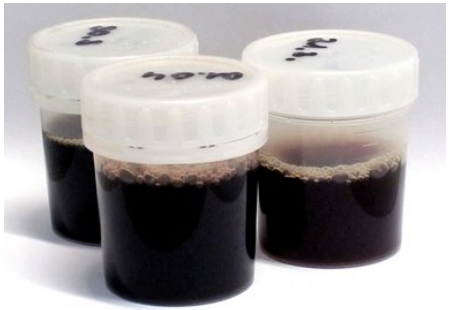
PNH - Symptoms

- **Intravascular hemolysis**
  - Nocturnal hemoglobinuria +- 25%
  - Anemia related symptoms
  - Vasospasm due to NO depletion
- **Thrombosis, typical and atypical localisations**
  - ! Leading cause of death !
  - Venous> arterial
  - Thrombotic risk correlates with clone size
- **Cytopenia/Bone marrow dysfunction**
  - BM dysfunction frequent
  - Overlap syndrom with AA/less common MDS



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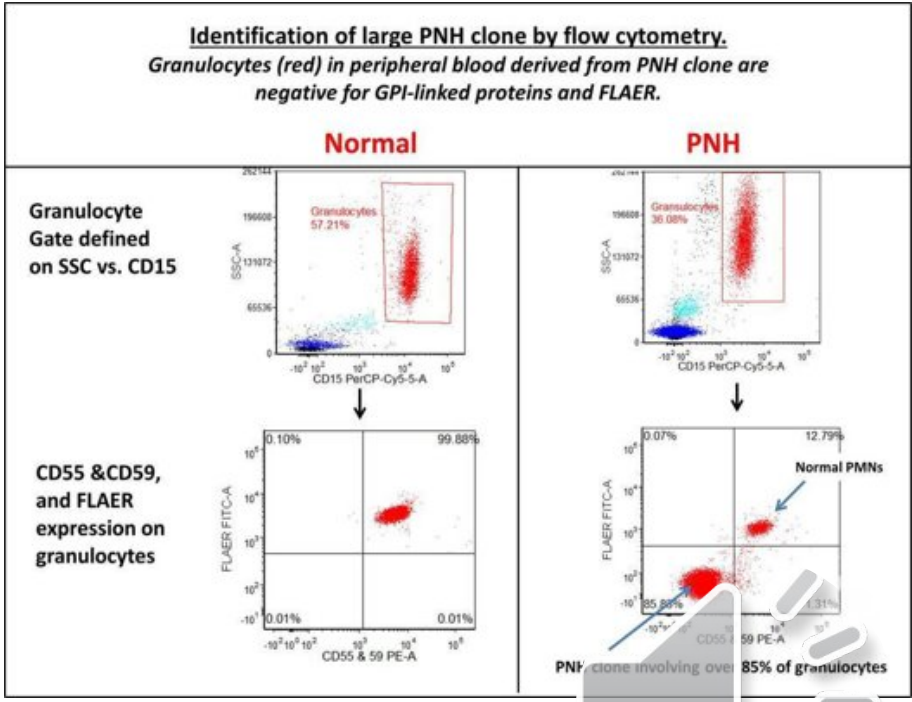
PNH - Diagnosis

- **Flow cytometry**

- Detect reduction or loss of GPI-anchored proteins on blood cells and define the size of the PNH population.



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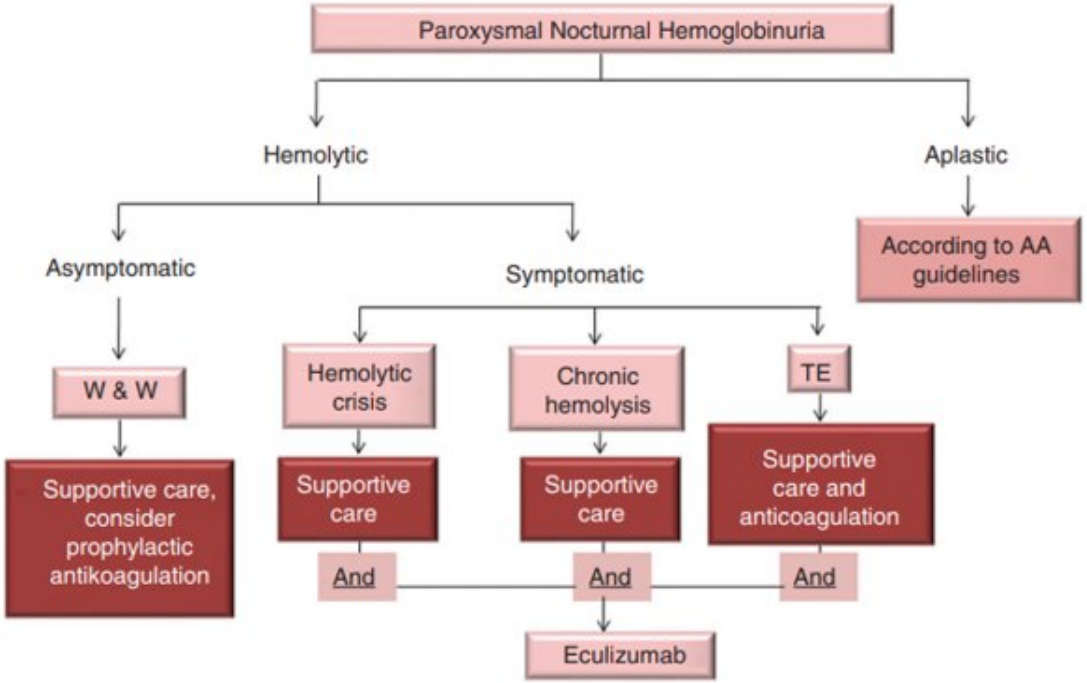
PNH - Treatment



Schrezenmeier et al, Treatment algorithm adopted from the German PNH guidelines

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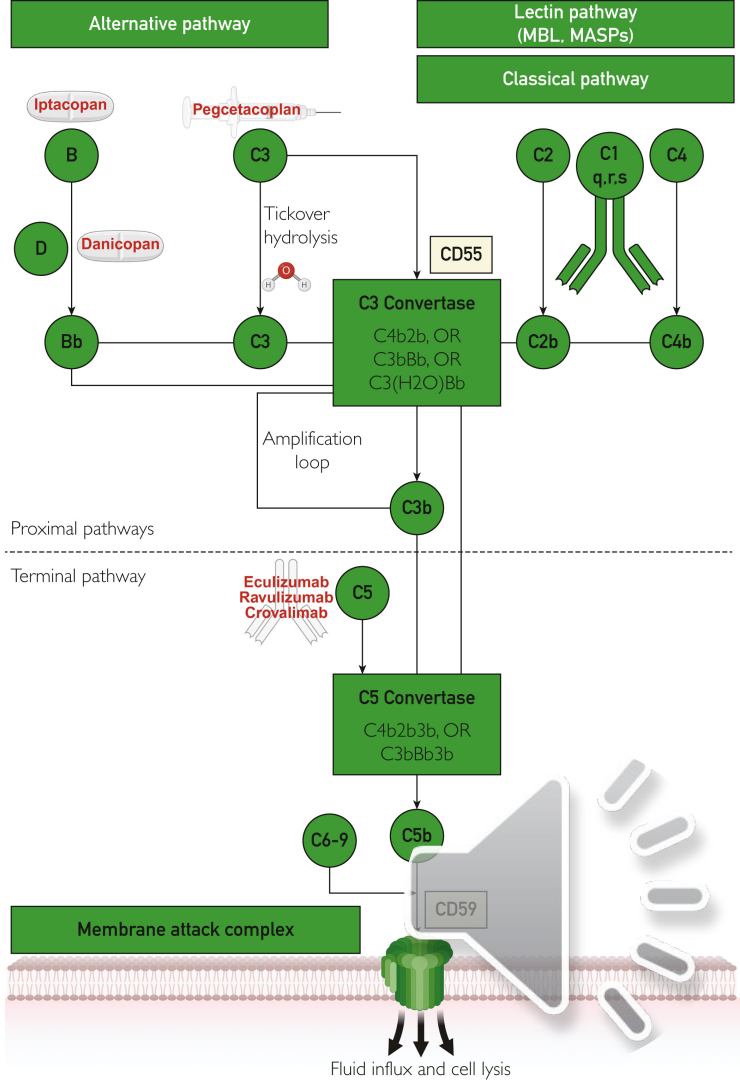
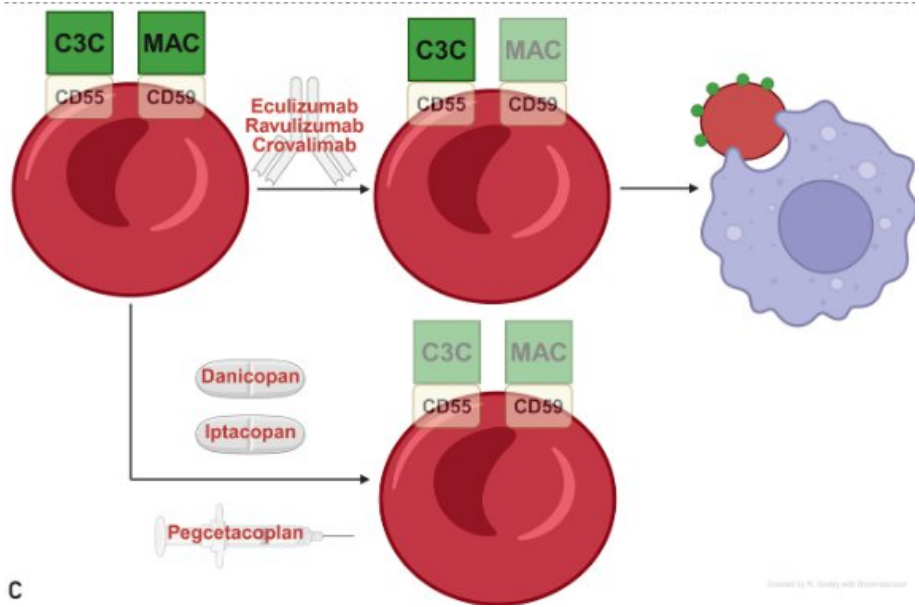
PNH - Treatment

- **Complement inhibitors**:
  - To reduce hemolysis
  - To reduce thromboembolic risk



Godby, Richard C. et al.
Mayo Clinic Proceedings,

PNH - Treatment



Godby, Richard C. et al.
Mayo Clinic Proceedings,

PNH - Treatment

Drug	Target	Schedule	Remboursement
Eculizumab	Anti-C5	600mg IV 1x/w for 4w 900mg IV 1x/2w	1st line
Ravalizumab	Anti-C5	IV, 1x/8w	1st line Switch form Eculizumab (stable case, after 6m)
Pegcetacoplan	Anti-C3	S.C., 2x/w	2nd line, after C5
Iptacopan	Anti-B factor	Oral, 200mg 2x/d	1st line 2nd line (persistent anemia, socio-economical)

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PNH - Treatment

- !! Risk of infection ☾ Vaccination against Neisseria meningitides
- Breakthrough haemolysis :
  - Pharmacokinetic : suboptimal plasma level until next administration ☾ reduce dose interval
  - Pharmacodynamic : Massive complement activation (infections)
  - Anti-C5 > anti-C3

Acquired Hemolytic Anemia

- Autoimmune hemolytic anemia (AHAI)
- Thrombotic microangiopathy (TMA)
- Paroxysmal Nocturnal hemoglobinuria
- **Hemolytic anemia and infections**
  - Parasites : plasmodium, babesia, leischmania
  - Bacterial : Clostridium Perfringens, Bartonella bacilliformis, Klebsiella pn,…
  - Sepsis
- **Transfusion hemolytic reaction**:
  - Acute (ABO mismatch)
  - Delayed (allo-immunization)

Thank you for your attention !

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