Hemolytic anemias
Anemias – Diff Dg Approach

Exclude blood loss

Reticulocyte count

- elevated > 2%
  - hemolysis
  - hemoglobinopathies

- low < 2%
  - hypoproliferative anemia

review peripheral blood smear
### Usefulness of the Reticulocyte (rtc) Count in the Diagnosis of Anemia

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypoproliferative anemias:</strong></td>
<td>Absol. rtc count &lt;75,000/μl</td>
</tr>
<tr>
<td>Anemia of chronic disease</td>
<td></td>
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<tr>
<td>Anemia of renal disease</td>
<td></td>
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<tr>
<td>Congenital dyserythropoietic anemias</td>
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<tr>
<td>Effects of drugs or toxins</td>
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<tr>
<td>Endocrine anemias</td>
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<tr>
<td>Iron deficiency</td>
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<tr>
<td>Marrow replacement</td>
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<tr>
<td>Hypoproliferative anemias: Maturation abnormalities</td>
<td>Absol. rtc count &lt;75,000/μl</td>
</tr>
<tr>
<td>Vitamin B₁₂ deficiency</td>
<td></td>
</tr>
<tr>
<td>Folate deficiency</td>
<td></td>
</tr>
<tr>
<td>Sideroblastic anemia</td>
<td></td>
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</tbody>
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Usefulness of the Reticulocyte (rtc) Count in the Diagnosis of Anemia

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<th>Diagnosis</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic anemias</td>
<td>Absol. rtc count &gt;100,000/μl</td>
</tr>
</tbody>
</table>

- Hemoglobinopathies
- Immune hemolytic anemias
- Infectious causes of hemolysis
- Membrane abnormalities
- Metabolic abnormalities
- Mechanical hemolysis
Hemolytic anemias

A. HEREDITARY

1. RED BLOOD CELL MEMBRANE DISORDERS
   - Hereditary spherocytosis, elliptocytosis
   - Acanthocytosis, Stomatocytosis

2. RED BLOOD CELL ENZYMES DISORDERS
   - non spherocytic hemolytic anemias - enzymes deficiency
     - G6PD (glucose-6-phosphate dehydrogenase deficiency)
     - PK (pyrivate kinanase deficiency)
     - other

3. DISORDERS of HEMOGLOBIN SYNTHESIS
   - hemoglobinopathy S, unstable Hb
   - thalassemias α, β
Hemolytic anemias

B. ACQUIRED

- AUTOIMMUNE HEMOLYTIC ANEMIA (AIHA)
- Paroxysmal nocturnal hemoglobinuria (PNH)
- Drugs
  - a/ penicilin type
  - b/ stibophen type
  - c/ alfphamethyldopa type
- Microangiopathic hemolytic anemia (MAHA sy)
- Chemical and physical causes
- Microorganisms
- methemoglobinemia, sulfhemoglobinemia
- hypersplenism
Hemolytic anemias

Pathophysiology remarks
Fig. 4.1 Haemolytic anaemia: extravascular and intravascular mechanisms of red cell breakdown.
**Hemolytic anemias**

**Definition:** premature destruction of erythrocytes - congenital or acquired

- **extravascular** = physiological process  
  (destruction of ery in macrophages - BM, spleen, liver)

- **intravascular** = pathological process

**Laboratory characteristic intravascular hemolysis**

- Hb
- reticulocytosis
- indirect bilirubin
- elevated LDH
- haptoglobin
Hemolysis and bilirubin

Extravascular or intravascular hemolysis

Blood

Hepatic sinusoid

Hepatocyte

Unconjugated bilirubin
transported with ligandin or Z protein
conjugated to glucuronic acid

Conjugated bilirubin

Urobilinogen

Biliary system

Portal vein

Small intestine

Kidney

Urobilinogen excreted in urine

Urobilinogen

10%

90%

Feces
Intravascular hemolysis

Haptoglobin in plasma
Intravascular hemolysis

hemoglobinuria
Interactions between membrane proteins of the erythrocyte forming membrane skeleton

The assembly of red cell cytoskeletal proteins form macro-complex (network) in the lipid layer
Hemolytic anemias

diagnostic approach
Hemolytic anemias - dg

Hb (➡)
rtc (➡)
peripheral blood smear - morphology
bilirubin, UBG v urine (➡)
free Hb in plasma (➡)
hemoglobinuria (➡)
haptoglobin (➡)
hemosiderin in urine (➡)
LD (LD1)
DAT , IAT (Coombs)

- **Special tests** (osmotic resistance, glycerin test (pink test); enzyme deficiency tests…
Direct antiglobulin test (DAT) for detection of (A) erythrocyte-bound C3d or (B) IgG.

Hemagglutination occurs when anti-C3d or anti-IgG can create a lattice structure by bridging sensitized red blood cells.
Indirect antiglobulin test for detection of antierythrocyte antibodies (Ab) in serum.

The patient's serum (with auto Ab) is mixed with normal rbc + anti-IgG reagent is added and hemagglutination occurs.
Hemolytic anemias

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   - other

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   - hemoglobininopathy S, unstable Hb
   - thalassemias $\alpha$, $\beta$
Hereditary spherocytosis

The typical features of HS:
- dominantly inherited hemolytic anemia
- anemia of mild to moderate severity
- spherocytosis on the peripheral blood film
- favorable response to splenectomy
- milder forms of HS might be asymptomatic

Prevalence of 1 in 2000 individuals

Ethiology

Hereditary defect of membrane protein synthesis $\rightarrow$ osmotic fragility $\rightarrow$ hemolysis
The assembly of red cell cytoskeletal proteins

(a) Red blood cell membrane

- Glycophorin A
- Band 3 tetramer
- RhAG
- CD47
- Rh protein
- Band 3 dimer
- Glycophorin C/D
- Protein 4.1R
- Adducin
- Spectrin self association site
- α Spectrin
- β Spectrin
- Nucleation site
- Dematin
- Actin
Selected ion pumps and passive diffusion of cations maintain the red cell volume and intracellular Na+/K+ gradients.

### Ion transport in red cells

```
<table>
<thead>
<tr>
<th>Cell</th>
<th>mmol/L cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>[K⁺]</td>
<td>100</td>
</tr>
<tr>
<td>[Na⁺]</td>
<td>8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Plasma</th>
<th>mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>[K⁺]</td>
<td>3.5–5.3</td>
</tr>
<tr>
<td>[Na⁺]</td>
<td>133–146</td>
</tr>
</tbody>
</table>
```

- **ATP dependent Na⁺/K⁺ pump**
- Long-term volume regulation with "passive" leak
- K⁺Cl⁻ co-transporter
- Minor role in short-term volume regulation; activated by cell swelling
Hereditary spherocytosis

HS pathophysiology

- dominantly inherited deficiency or dysfunction of proteins of the erythrocyte membrane leads to a multistep process of accelerated HS red blood cell destruction

- poorly deformable spherocytes are selectively retained and damaged in the spleen

- splenic vasculature acts as a microcirculation filter
Spherocytes: characteristic shape
- lack central pallor
- mean cell diameter is decreased
- they appear more intensely hemoglobinatinated

(which reflects both altered red blood cell geometry and increased cell density)
Hereditary spherocytosis

*Molecular Pathology*

The molecular basis of HS is heterogeneous.
(by densitometric quantitation of membrane proteins)

1) isolated deficiency of spectrin
2) combined deficiencies of spectrin and ankyrin
3) deficiency of band 3 protein
4) deficiency of protein 4.2
5) no abnormality identified
Hereditary spherocytosis

Clinical Manifestations

Typical Forms - relatively asymptomatic
- mild jaundice …. „nice tan“…
- splenomegaly gradually develops in most pts.
- anemia is usually mild to moderate
- occasionally anemia is absent, the reticulocyte count is normal or only minimally elevated, laboratory evidence of hemolysis is minimal or absent

Severe and Atypical Forms
- severe life-threatening hemolysis early in life
- Transfusion dependent during early infancy and childhood
- Activation asymptomatic HS: Parvovirus B 19, HS 6, CMV, pregnancy, but postpartum pts conditions return to baseline level
Complications:

**Bilirubin gallstones** - 50% of pts with HS

**Hemolytic crises** – reticulocytes

= hemolysis and accelerated erythropoiesis

**Aplastic crises** - reticulocytes - 0 !!

= hemolysis and stop of erythropoiesis

- (Parvovirus B 019 - the virus selectively infects erythroid precursors and inhibits their growth)
Clinic: splenomegaly
Labor: Hb ↓, MCV ↓, MCHC ↑, RDW ↑, rtc ↑
Blood smear: morphology - spherocytes
Coombs test: negat
Hemolytic picture: indirect bilirubin, rtc
RBC haptoglobin: normal
autohemolysis: 48 h incubated without glu 10-50% (N: 4%)
(after glucose is added)
Bone marrow: hypoplasia – aplasia - hyperplasia

**Advanced: Detection of the Molecular Defects**
- Gel elektrophoresis (= analysis of membrane proteins)
- Genetics (gen mutation for β-spectrin, protein 4.2…)
- **Screening:** EMA test: binding of eozin-5-maleimid to protein band 3 is weakened
Hereditary spherocytosis - therapy

Splenectomy = curative

Indications for splenectomy:
- growth retardation, skeletal changes
- symptomatic hemolytic disease
- anemia-induced compromise of vital organs
- the development of leg ulcers
- appearance of extramedullary hematopoietic tumors
Hemolytic anemias

B. ACQUIRED

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  a/ penicilin type  
  b/ stibophen type  
  c/ alfphamethyldopa type
- Microangiopathic hemolytic anemia (MAHA sy)
- Chemical and physical causes
- Microorganisms
- methemoglobinemia, sulfhemoglobinemia
- hypersplenism
Autoimmune Hemolytic Anemia (WARM AIHA)

Definition:
AIHA - spectrum of disorders – antibodies against self-antigens on the ery membrane – hemolysis –
- can occur as an idiopathic (primary) disorder or
- can coexist with another disease (secondary)

3 types:
1/ associated with warm-reactive IgG autoAb
   warm autoimmune hemolytic anemia (WAIHA)
2/ hemolytic anemias caused by cold-reactive IgM autoAbs
cold agglutinin disease
3/ sy associated with the Donath-Landsteiner antibody as
   paroxysmal cold hemoglobinuria

Incidence: 1/ 80 000 / year
### 3 types of Autoimmune Hemolytic Anemia

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Warm AIHA</th>
<th>Cold Agglutinin Disease</th>
<th>Paroxysmal Cold Hemoglobinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody isotype</td>
<td>IgG, rare IgA, IgM</td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>Direct antiglobulin test (DAT) result</td>
<td>IgG and/or C3</td>
<td>C3</td>
<td>C3</td>
</tr>
<tr>
<td>Antigen specificity</td>
<td>Multiple, primarily Rh, i/l, Pr, P</td>
<td>Primary extravascular</td>
<td>Intravascular</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>Primarily extravascular</td>
<td>Primary extravascular</td>
<td></td>
</tr>
<tr>
<td>Common disease associations</td>
<td>B-cell neoplasia /lymphoproliferative, collagen–vascular</td>
<td>Viral, neoplasia</td>
<td>Syphilis, viral</td>
</tr>
</tbody>
</table>
AUTOIMMUNE HEMOLYTIC ANEMIAS

1/ WARM AUTOIMMUNE HEMOLYTIC ANEMIA

Warm autoantibodies = IgG
Optimal binding to rbc = 37°C
Both serious clinical picture and laboratory findings

2/ Cold autoantibodies = IgM
Optimal binding to rbc = 4°C, start 20°C
Usually normal clinical picture and serious laboratory

3/ Mixed
Mechanism of extravascular hemolysis in autoimmune hemolytic anemia

Macrophage encounters an IgG-coated erythrocyte and binds to it via its Fc receptors

Light coat (no complement) → Splenic removal (slow circulation)

Heavy coat (plus complement) → Hepatic removal (fast circulation)
<table>
<thead>
<tr>
<th>Collagen vascular disease</th>
<th>Miscellaneous diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>Myelofibrosis with myeloid metaplasia</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>Ulcerative colitis</td>
</tr>
<tr>
<td>Polyarteritis nodosa</td>
<td>Pernicious anemia</td>
</tr>
<tr>
<td>Serum sickness</td>
<td>Thyroid disease</td>
</tr>
<tr>
<td>Sjögren syndrome</td>
<td>Ovarian cysts</td>
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<thead>
<tr>
<th>Lymphoreticular malignancy</th>
<th>Miscellaneous diseases</th>
</tr>
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<tbody>
<tr>
<td>Macroglobulinemia</td>
<td>Evans syndrome</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>Congenital immunodeficiency syndromes</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>Guillain-Barré syndrome</td>
</tr>
<tr>
<td>Mycosis fungoides</td>
<td>Primary biliary cirrhosis</td>
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</table>

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<tr>
<th>Other malignancy</th>
<th>Miscellaneous diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute leukemia, Carcinoma (colon, kidney, lung, ovary), Thymoma</td>
<td>Multiply transfused pts with Hb-pathies</td>
</tr>
</tbody>
</table>
AIHA - clinical findings

**Disease can occur:**

*acutely*, with symptoms caused by rapidly developing anemia – *overt anemic syndrome*

*gradually* in a relatively asymptomatic form – *mild anemia*

*occasionally = asymptomatic form*, the blood bank provides the diagnosis through a positive antiglobulin test

When to start a search for an underlying disease –

*secondary AIHA*: lymphadenopathy, fever, hypertension, renal failure, rash, petechiae, or ecchymoses…
AIHA - laboratory evaluation

Blood picture: anemia macrocytic, \( \uparrow \) rtc

Biochemistry \( \uparrow \) bili, UBG in the urine

Immunohematology: DAG, IAT,

Basic characteristic of hemolysis - summary:
- \( \downarrow \) Hb
- \( \uparrow \) retikulocytosis
- \( \uparrow \) indirect bili
- \( \uparrow \) LDH
- \( \downarrow \) haptoglobin
AIHA Therapy - General Principles

AIHA ranges from indolent to life threatening clinical course

Evaluation

AIHA is primary or secondary

Initiation of treatment

- Treatment of underlying disease
- Immediate transfusion
- Attempts to suppress the production of autoantibodies
Some cases of AIHA are life threatening and necessitate transfusion with RBCs

- autoantibodies complicate the pretransfusion evaluation

- despite a complete blood bank workup, the patient often will receive “cross-match incompatible” blood

- In situations where transfusion is required, blood should not be withheld simply because it is not fully compatible.

- However, transfusion should be administered with particular care and close monitoring

- Transfusion is warranted without delay and, if necessary, before all serologic tests are completed when cardiac or cerebral function is threatened
AIHA - therapy summary

1/ corticosteroids **PREDNISON** 1-2 g i.v./day x 5 days, then dose reduction to 1 mg/kg… ASAP

2/ **IMMUNOMODULATORY THERAPY** (Cyclof., Azathioprine …)

3/ **IVIG** 0,1-1,0 g /kg /day

4/ **PLASMAPHERESIS**

5/ **RITUXIMAB** (anti CD20 monoklonální protilátka)

6/ **SPLENEKTOMIE**

Trf ery - vitální indikace - pečlivý monitoring vitálních funkcí - zajištění kortikoidy ….
1/ AIHA Therapy - Corticosteroids

- **first-line** - either the idiopathic or the secondary form (to disable macrophages from clearing IgG, inactivated C3b, or C3b-coated rbc)

- **begin with prednisone** (there is no clear advantage to alternative forms of corticosteroids) **1 - 2 mg/kg/day** (in elderly 0.6 mg/kg/day)

- it should be **continued until** a response becomes evident, usually within **3 weeks**

  . **Until** - Hb level reaches **≥ 100 g/l**
    - transfusion should no longer be necessary

  . **Thereafter:**
    - the dose can be gradually **reduced**
    - usually at a rate of **5 to 10 mg/week**
AIHA - Immunomodulatory Therapy

- alkylating agents - 3 months or more
  - Cyclophosphamide 2 mg/kg/day
  - chlorambucil
- thiopurines
  - Azathioprine 1.5 mg/kg/day
  - 6-mercaptopurine
- Enables substantially reduced doses of prednisone
- Immunosuppressants:
  - Cyclosporine alone and in combination
  - 2.5 mg/kg twice daily for 6 days then 3 mg/kg/day and 5 mg/day of prednisone
3/ AIHA Therapy - *Intravenous IgG*

**IVIG 0.1-1.0 g/kg/day**
(The soluble IgG in the material may increase the lifespan of IgG-coated RBCs by saturating Fc receptors on macrophages)

- for treatment of **severe life-threatening AIHA**
- in cases where AIHA is **refractory to conventional treatment with corticosteroids**
- temporizing measure **prior to splenectomy**
- **not recommended for routine use** in either acute or chronic treatment of AIHA
4/ AIHA Therapy - *Plasma Exchange*

- the therapeutic advantage - **removal antibodies**

- Not long-term efficacy (continuous Ab production and the large extravascular distribution of IgG in pts with IgG-mediated AIHA)

- on cessation of therapy - return to pretreatment levels

- this therapy should be **reserved for**:
  - patients **unresponsive to trf**
  - pts in **critical condition** (rapid clearance of rbc)
  - **before splenectomy**
AIHA Therapy - Rituximab Therapy

- chimeric anti-CD20 monoclonal antibody = MabThera
  - regimen: 1xc/week x 3 weeks
- mechanism of action - multifaceted, complex
- induces cell death through complement-dependent lysis;
- antibody-dependent cellular toxicity
- antibody-dependent phagocytosis mediated by Fc, complement, and phosphatidylserine receptors
- direct antibody effects of CD20 ligation leading to inhibition of proliferation
- apoptosis and sensitization to chemotherapy
- and induction of active immunity
Proposed mechanisms of action of MabThera
6 AIHA Therapy - splenectomy

Indications
- failure to respond to prednisone, dosages ≥ 20 mg/day
- or intractable side effects of the corticosteroid

Highly effective
- by removing the major RES site of RBC destruction
- eliminates many phagocytosing macrophages
- and autoantibody-producing B cells

Risk
- sepsis by encapsulated organisms (Pneumococci)
  = immunization with pneumococcal and meningococcal vaccines,
EXTRINSIC NONIMMUNE HEMOLYTIC ANEMIAS

FRAGMENTATION HEMOLYSIS:
MICROANGIOPATHY

Thrombotic microangiopathy (TMA).

Thrombotic thrombocytopenic purpura–hemolytic uremic syndrome (TTP–HUS)

siamese siblings
Red Blood Cell Fragmentation Hemolysis

Damaged microvasculature
Thrombotic thrombocytopenic purpura–hemolytic uremic syndrome (TTP–HUS)

Associated with pregnancy:
Preeclampsia or eclampsia;
HELLP syndrome (hemolysis plus elevated liver enzymes plus low platelets)

Associated with malignancy, with or without mitomycin C treatment
Vasculitis: Polyarteritis, Wegener granulomatosis, acute glomerulonephritis, or Rickettsia-like infections

Abnormalities of renal vasculature:
Malignant hypertension, acute glomerulonephritis, scleroderma, or allograft rejection, with or without cyclosporine treatment
Disseminated intravascular coagulation
Malignant hypertension
Catastrophic antiphospholipid antibody syndrome

Atrioventricular malformations
Kasabach–Merritt syndrome
Hemangioendotheliomas
Atrioventricular shunts for congenital and acquired conditions (e.g., stents, coils, transjugular intrahepatic portosystemic shunt, Levine shunts)

Cardiac abnormalities
Replaced valve, prosthesis, graft, or patch
Aortic stenosis or regurgitant jets (e.g., in ruptured sinus of Valsalva)

Drugs: Cyclosporine, mitomycin, ticlopidine, tacrolimus, or cocaine
Clinical spectrum of thrombotic microangiopathy

- Haemolytic uremic syndrome
- Thrombotic thrombocytopenic purpura
- Malignant hypertension
- Preeclampsia – eclampsia
- Systemic lupus erythematosus
- Antiphospholipid antibody syndrome
- Systemic sclerosis
- Transplant associated
- Radiation therapy associated
- Drug induced
- Infection associated
Acquired hemolytic anemias non-immune

TTP – HUS Thrombotic thrombocytopenic purpura–hemolytic uremic syndrome

abnormally decreased ADAMTS-13 activity (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats

- metalloprotease - converting the highly thrombogenic large multimers of von Willebrand factor made by platelets and endothelial cells into the smaller – attached to endothelial cell surfaces, where platelets may excessively aggregate, leading to formation of microvascular thrombi even in the absence of endothelial damage + fibrin fibres – damaged rbc = schistocytes.

ADAMTS13 deficiency: 1/ acquired (autoAb)

2/ hereditary
TTP – HUS Thrombotic thrombocytopenic purpura
Hemolytic uremic syndrome

= ADAMTS-13 Metalloprotease (MP) deficiency:

MP cleaves the highly thrombogenic large multimers vWF, made by platelets and endothelial cells into the smaller...attached to endothelial cell surfaces...

missing MP → formation of large multimers vWF → → microvascular thrombi + fibrin fibres → → damaged rbc = schistocytes = hemolysis = manifestation of TTP.
Pathogenesis of idiopathic thrombotic thrombocytopenic purpura: idiopathic TTP caused by ADAMTS13 deficiency due to gene mutations or autoantibodies. Multimeric VWF adheres to endothelial cells or to connective tissue exposed in the vessel wall. Platelets adhere to VWF through platelet membrane GPIb. In circulation, VWF is unfolded by shear stress and cleaved by ADAMTS13, limiting thrombus growth. If ADAMTS13 is severe deficiency, accumulation of VWF-platelet aggregation continues, eventually causing microvascular thrombosis and TTP.
schistocytes
HUS - classic triad

- Microangiopathic anemia
- Thrombocytopenia
- Acute renal failure

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Diagnostic tools</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemolysis</td>
<td>Haemoglobin ↓  red blood cells ↓</td>
</tr>
<tr>
<td></td>
<td>Reticulocytes ↑</td>
</tr>
<tr>
<td></td>
<td>Lactate dehydrogenase ↑</td>
</tr>
<tr>
<td></td>
<td>Haptoglobulin ↓  free serum haemoglobin ↑</td>
</tr>
<tr>
<td></td>
<td>Direct antiglobulin test (Coombs test): negative</td>
</tr>
<tr>
<td></td>
<td>Schistocytes ↑</td>
</tr>
</tbody>
</table>
Laboratory findings:
- **Anemia** + rctytosis + **schistocytes**↑ akanto- sphero
- **trombocytopenia** – DIC
- elfo vWF - multimers (TTP)
- molecul. genet **ADAMTS13 Ag deficit**; **Coombs negat** !!
- ↑ Bili ↑ **LD**, ↓ **haptoglobin**
  ↑ proteinuria, ↑ Hb-uria, ↑ free Hb, ↑ creatinin, urea

**Therapy** TTP/HUS
  - FFP + exchange plasmapheresis, corticosteroids, anticoagulants, trf RBC, Plts
Untill TTP is not excluded, the TTP must be considered

1/ urgent

a/ Plasma (exchange) therapy (FFP) (30-50 ml/kg/day)
   replace ADAMTS13 + remove antibodies

b/ Immunosuppression (methylprednisolone 2mg/kg/den iv.)

c/ eculizumab

d/ thrombocytes and rbc supplementation

e/ CVVH – renal failure

f/ anticoagulation (DIC)
Paroxysmal Nocturnal Hemoglobinuria
Paroxysmal Nocturnal Hemoglobinuria
Triad of Clinical Features

Haemoglobinuria

- Intravascular haemolysis
  - disabling symptoms
    - abdominal pain
    - dysphagia
    - erectile failure
    - severe lethargy

Budd-Chiari syndrome

- Thrombosis
  - liver, cerebral
  - 50% of patients
  - 33% of patients is fatal

Aplastic anaemia

- Bone Marrow Failure
  - often precedes PNH
  - selects for PNH clone
Proteins Deficient from PNH Blood Cells

CD55, CD58, CD59, PrPC, AChE, JMH Ag, Dombroch, HG Ag

CD55, CD58, CD59, CD109, PrPC, GP500, Gova/b

CD55, CD58, CD59, CD109, PrPC, ADP-RT

CD55, CD58*, CD59, CD48, CD73, CD108

CD55, CD58, CD59, CD109, PrPC, GPI

CD55, CD58, CD59, CD109, PrPC, CD16

CD14, CD55, CD58*, CD52, CD157, PrPC, GPI-80

(Courtesy of Lucio Luzzatto)
Relative Growth Advantage in PNH

Normal stem cells

GPI-deficient (PNH) stem cells

Immune attack via GPI-linked antigen (aplastic anaemia)
PNH is a Progressive Disease of Chronic Haemolysis (1-4)

Normal red blood cells are protected from complement attack by a shield of terminal complement inhibitors (2,3).

Without this protective complement inhibitor shield, PNH red blood cells are destroyed (2,3).

Complement Activation

Intact RBC

Free Haemoglobin in the Blood from Destroyed PNH RBCs

Anaemia

Erectile Dysfunction

Significant Impact on Survival (3)

Thrombosis

Renal Failure

Pulmonary Hypertension

Abdominal Pain

Dyspnoea

Fatigue

Dysphagia

Haemoglobinuria

Significant Impact on Morbidity (3)

Chronic Haemolysis is the Underlying Cause of Progressive Morbidities and Mortality of PNH (1-5)

- **THROMBOSIS** (2,4,5)
  - Venous
    - PE/DVT
    - Stroke/TIA
  - Arterial
    - Cerebral
    - MI
    - Dermal
    - Hepatic/Portal
    - Abdominal ischemia

- **Pulmonary Hypertension** (3,4)
  - Dyspnoea
  - Cardiac Dysfunction

- **Fatigue / Impaired Quality of Life** (3,4)
  - Abdominal pain
  - Dysphagia
  - Poor physical functioning
  - Erectile dysfunction

- **Chronic Kidney Disease** (3,4)
  - Renal insufficiency
    - Dialysis
    - Anaemia

- **End Organ Damage** (2,3,4)
  - Brain
  - Liver
  - GI

- **Anaemia** (2,4,5)
  - Transfusions
  - Haemosiderosis

References:
- (2) Hillmen P et al. NEJM 1995;333:1253-58
- (3) Rother R et al. JAMA 2005;293:1653-62
Renal Damage in PNH

Chronic haemolysis and cell-free plasma haemoglobin lead to chronic kidney disease in PNH (1,2)

Renal damage in PNH may be due to repetitive exposure of tissue to cell-free haemoglobin (3,4)

64% of patients with PNH have stage 1-5 chronic kidney disease (5)

Renal failure has been identified as the cause of death in approximately 8 – 18% of PNH patients (6,7)

Routine Red Cell Analysis: Reagents

- For historical reasons, CD55 and CD59 are most commonly used
- CD59 is strongly expressed, while CD55 is weak
  - CD55 may not be necessary
  - Rare congenital CD59 deficiency cases
  - Some variation in CD59 clones
- Other GPI-anchored reagents (CD58) exist, but limited experience
- Anti-glycophorin (CD235a) may be used to identify red cells, but this may not be necessary for routine analysis
  - Can guard against failure of antibody to contact cells
Leucocyte Analysis: Routine testing

- Granulocyte PNH clone probably gives most accurate estimate of PNH clone size
- Monocyte clones can usually be determined in same tube and confirms granulocyte result, though because monocytes are less numerous, precision is lower
- Type II granulocytes can occasionally be recognized but red cells are typically better for this purpose
- Lymphocytes are not a suitable target for testing
WHAT IS FLAER?

**FL**uorescent **AERolysin**

Aerolysin is a pore-forming toxin secreted by *Aeromonas hydrophila* GPI-anchor serves as receptor

FLAER – A488-conjugated mutant aerolysin binds to GPI-anchor rather than surrogate protein and is inactive so doesn’t form channels